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THE LIFE CYCLE OF TWO SPECIES OF "*SELENIDIUM*" FROM THE POLYCHAETE WORM *POTAMILLA RENIFORMIS*.

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(With Plates XIII–XVII, containing Figs. 1–38, and Figs. I–XVIII in the Text[1].)

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I. INTRODUCTION.

WE have written the word "*Selenidium*" in inverted commas, for, as one of us (Ray, 1930 *a*) has shown, the gregarines classed as species under this name behave very variously, and the probability is that the genus will have to be dismembered.

Giard (1884) created the genus *Selenidium* for a nematode-like gregarine, *S. pendula*, in the gut of a species of *Nerine*; and a number of similarly shaped gregarines showing active wriggling movements and occurring in the gut of other polychaetes and of some Gephyreans have since been described by protozoologists as species of *Selenidium*. *Gregarina spionis* and *G. terebellae*,

[1] N.B. Figures on plates bear *Arabic* numbers whilst figures in the text bear *Roman* numbers.

described by Kölliker as early as 1845, were also organisms of this kind. Mingazzini (1891) may have been justified in re-naming the second of these *Esarhabdina terebellae*, but only a study of the life cycle will determine this; morphologically, the trophozoite of the gregarine from Terebella is a "*Selenidium*."

Caullery and Mesnil (1899 and 1900) were the first to attempt a study of the life history of *Selenidium*. They not only described a number of species (one of which, as Ray has shown, was undoubtedly identical with Kölliker's *Gregarina spionis*); they noted association, the peculiar method of formation of the gamete nuclei, and, in *S. echinatum* from *Dodecaceria concharum*, they found the ripe spores. These spores were rough coated and contained four sporozoites each. No one else ever found the spores of *Selenidium* until Ray (1930 *a*) discovered that the gametocysts of the two species inhabiting *Scolelepis fuliginosa* (*Selenidium spionis* and *S. foliatum*) produce spores in sea water after leaving the body of the host. The sporocysts of these species are smooth walled, and, strange to say, while those of *S. spionis* contain four sporozoites, those of *S. foliatum* contain eight[1].

Selenidium is classed among the schizogregarines. Caullery and Mesnil (1901) claimed to have found the intracellular schizogonic phase of one of the species in *Scolelepis fuliginosa*; but Ray has since shown that these authors confused with their gregarine's life cycle the schizogony of a coccidian-like organism living alongside (Ray, 1930 *b*). Brasil (1907) demonstrated intracellular schizogony of a very different type in *Selenidium caulleryi* from *Protula tubularia*; this was also seen by Ray, who considers that Brasil was fully justified in his conclusions. Brasil and Fantham (1907) also published a note on intracellular schizogony in one of the species of *Selenidium* from *Phascolosoma*, but they gave no figures. From 1907 onwards, an intracellular schizogonic phase was stated to be the chief characteristic of the genus *Selenidium* among schizogregarines, and such is now the definition given in textbooks of protozoology.

A more extensive study of *Selenidium*-like organisms showed Ray that another of these gregarines from polychaetes, *Selenidium mesnili* Brasil, also undoubtedly conforms to the modern definition. But in no other species that he examined could he discover any trace of schizogony; and he further observed that, while some are intracellular for the greater portion of their lives as parasites in the gut, others are intracellular for only a brief period after their emergence from the spore, while some seem to be free in the gut lumen from first to last.

Facts like these have led us to doubt whether the species are really so closely related as their association under one generic name would suggest. It is obviously important to discover what is the behaviour of the type species, *Selenidium pendula* Giard. Has this organism an intracellular schizogonic

[1] In this laboratory Miss N. Reed, working on the sporogony of *Selenidium mesnili*, has observed the spores in that species also. She finds four sporozoites in each sporocyst.

phase, and how many sporozoites do its sporocysts contain? Unfortunately, we have not found *S. pendula* in *Nerine foliosa* Aud. and Edw. at Plymouth, and Giard does not state what was the species of *Nerine* he examined. But we have continued our investigation of the life cycle of similar gregarines, and we are now in a position to state with some precision what happens to those two species which habitually parasitise the gut of the polychaete, *Potamilla reniformis* (O.F.M.), at Plymouth[1].

While meantime leaving the smaller of these in the genus *Selenidium* as *S. potamillae* n.sp., we feel justified in beginning the dismemberment of *Selenidium* Giard by creating for the larger one a new genus, *Meroselenidium*, with *M. keilini* from *Potamilla* as the type species.

II. MATERIAL AND METHODS.

All the worms came from Plymouth, and were examined by us either there or in a London laboratory, where they lived quite well for a time in aerated sea water. *Potamilla* lives gregariously in brown, leathery, interwoven tubes, and it is not surprising that the rate of infection should be very high[2]. The worms were removed from their tubes, narcotised, and then cut into pieces which were separately embedded and sectioned after samples of the gut contents had been examined as the fluid oozed away from the cut ends.

The parasites were always studied alive and in their natural fluid medium. But we found that the occasional addition of sea water produced no ill effects on them whatsoever.

We used a variety of fixatives, but in the end depended almost entirely on Brasil's modification of Bouin-Duboscq's fluid. We fixed some in Helly's fluid for study of the cytoplasmic inclusions. Dobell's modification of alcoholic Bouin was the best fixative for the sporocysts, though Carnoy also proved useful.

The stains employed were Mayer's acid haemalum, Delafield's haematoxylin, Dobell's modification of Mann's methyl-blue-eosin, Altmann's acid fuchsin, and Heidenhain's haematoxylin counter-stained by various dyes, of which chromotrop 2R gave the best results. The most successful stains for the sporocysts were Delafield counter-stained with chromotrop, and carbol-fuchsin with picro-nigrosin. Sections were usually cut 8μ thick; though for the sporocysts we sometimes cut as thin as 4μ.

To obtain ripe gametocysts, we took the worms from their tubes, washed them well in tap water, dipped them in sea water that had been filtered through bolting silk, and then put them in a glass vessel of sea water similarly filtered, which we kept in a cool place under constant aeration.

[1] The only other sporozoan parasite of *Potamilla* to which we find any reference is *Haplosporidium potamillae* Caullery and Mesnil, in the body cavity of *P. torelli*.

[2] Every worm examined was parasitised more or less heavily with *Meroselenidium*, and 50 per cent. showed *Selenidium potamillae*.

III. Description of the gregarines.

A. *Meroselenidium keilini* n.gen., n.sp.

(1) *Schizogony.*

We have abundant evidence that in this gregarine, as in *Selenidium mesnili* and *S. caulleryi*, the large forms found free in the gut lumen are the product of schizogony, and that this asexual multiplication is usually intracellular, though apparently the schizonts may sometimes lie between cells.

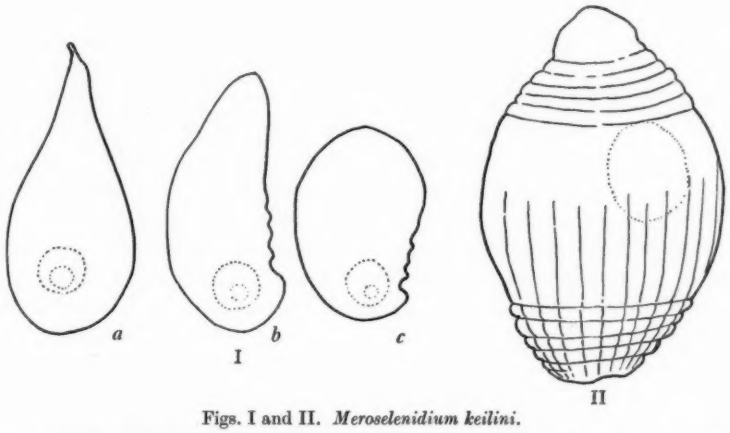
The schizonts are to be found in the wall of the anterior portion of the middle stretch of the intestine. They are never numerous in any one worm; but they grow to a great size and produce such vast numbers of merozoites that even a few will serve as a stock sufficient to account for the swarm of free gregarines further back in the gut.

We have never found the invading sporozoites from which presumably the schizonts develop. The smallest schizonts that we have seen already measure $50 \times 18\mu$ (Fig. 1). These asexual individuals usually lie in epithelial cells: frequently two occur side by side. The nucleus at this stage is relatively enormous. It contains two karyosomes: one of these is very much larger than the other, and, as the parasite grows, it tends to stain more faintly or to become vacuolated. When the parasite is intracellular, its long axis is parallel to that of the cell; where, as occasionally happens, it has penetrated deeper and lies below the epithelium, its long axis is at right angles to those of the cells (Fig. 2). A full-grown schizont measures about $150 \times 30\mu$; but the exact size is difficult to determine from sections, since confinement within the tissue forces the elongating body to become transversely crumpled and its ends tend to curve over (Fig. 3). The inner, presumably the anterior, end tapers to a narrow neck region. The surface of the body is bounded by a pellicle marked with about thirty longitudinal furrows and flattened ridges, which converge fore and aft: these striations are not obvious in the younger specimens. Occasionally in sections of large schizonts we could see that a system of transversely disposed lines lies below the pellicle, showing up more clearly in the furrows (Fig. 4): the gregarine is, however, almost immobile at this stage, and the function of such myonemes—if myonemes they be—is not obvious. The large schizonts are inert, opaque, pear-shaped bodies (Fig. I, *a*, *b* and *c*) which, when artificially liberated, soon shorten and thicken until they have assumed a spherical form. The pellicle appears to be very thin; it shows the longitudinal striations which can be seen in sections. As the body assumes the spherical shape, transverse wrinkles appear on its surface, especially towards the poles (Fig. II). Fixed and stained smears of some of these liberated schizonts do not reveal the internal structure as well as do the sections.

In a schizont measuring $120 \times 30\mu$ the nucleus is still 15μ in diameter; occasionally a few siderophilous granules lie scattered in the cytoplasm. We have not been able to trace the division process through the early stages. In

one specimen (Fig. 5) we saw some minute nuclear spindles and a few faintly staining resting nuclei; but the great majority of dividing schizonts show the surface already covered with very small dense nuclei.

Very characteristic of the asexual multiplication in this gregarine is the preliminary division of the cytoplasm within the pellicle into a number (usually eight or ten) of sub-spherical masses, each measuring about 18μ in diameter and studded all over with nuclei (Fig. 6). It is possible that these masses are comparable with the cytomeres formed by the schizont of the coccidian *Caryotropha mesnilii* (see Siedlecki, 1907), each at first uninucleate and then proceeding independently to merozoite formation. Or it may be that in *Meroselenidium*, as in the coccidian *Aggregata eberthi*, there is abundant superficial folding of the schizont, of which the "cytomeres" here are the ultimate



Figs. I and II. *Meroselenidium keilini.*

Fig. I. Freehand drawings of the living freed schizont. *a*, *b* and *c* show successive stages in its shortening and thickening. × 680.

Fig. II. A larger schizont under higher magnification, showing the longitudinal striations of the pellicle and the terminal transverse wrinkling. × 800.

expression. In the schizogregarine, the spherical masses become completely separated from one another; and, since each produces merozoites over its entire surface, the reproductive powers of the schizont are enormously increased.

Exterior to each small nucleus, the cytoplasm becomes raised in a slight prominence, and, as this lengthens to give the merozoite, the nucleus moves into it. By this time each cytomere has a dense covering of merozoites: in the centre of the group lies a residual mass (Fig. 7). All the cytomeres are still enclosed within the pellicle of the schizont.

Before their liberation the merozoites become somewhat curved and flattened. The ring-like nucleus lies in the proximal third, and between the nucleus and the distal end three to five dark-staining lines have developed; of

these lines, one is often longer than the others (Fig. 8). The merozoites now break away and travel outwards into neighbouring cells; the pellicle of the schizont and the residual cytoplasmic bodies seem to be absorbed; but the host cell forms by this time so frail an envelope that its rupture can easily take place, and not infrequently we have found groups of merozoites free in the lumen of the gut. Their escape in this way may be facilitated by the abrading action of diatoms and sponge spicules passing in the worm's food.

We have never seen the merozoites alive. After entering a cell, the merozoite becomes surrounded by a vacuole and proceeds to grow. When it is about $14 \times 4\mu$ its anterior end has become blunt; at this stage the nucleus generally shows three or four dark granules lying together in the centre, separated by a clear zone from the membrane. On the surface of the anterior, now the more obtuse end of the parasite, the dark striations can still be seen; but they have shortened and thickened (or else their posterior ends have faded away) (Figs. 9 and 10). Figs. 11 and 12 show later stages in growth: the parasites seem to remain within the epithelium until they are about 50μ in length. Occasionally we have found much smaller individuals free in the gut lumen (Fig. 13), but probably these have developed from merozoites liberated directly into the cavity as described above. The nucleus of the imprisoned parasite is usually of the form shown in Fig. 12; it is much compressed, and the chromatic contents are now arranged in one (sometimes two) densely staining karyosome and a clump of paler granules alongside. In some of the larger parasites one can see that the pellicle has thickened and shows signs of longitudinal striation. Since many adjacent cells are infected, a sort of tumour is formed; the host cells degenerate and this must facilitate the emergence of the gregarines.

Once out in the gut, the parasites migrate further into its posterior half and attach themselves to the surface of the epithelium. They now grow to a great length and develop a powerful myoneme system which enables them to perform the wriggling movements so characteristic of selenidia. In some worms the gregarines are present in such enormous numbers that the passage of the gut is almost blocked by their writhing bodies (Fig. III).

(2) *The adult trophozoite.*

The adult gregarine (Fig. 14), free in the lumen of the gut, measures $200–300 \times 40–70\mu$. The widest part of the body is a short way behind the anterior end, and on one surface is convex, on the other flattened or slightly concave (Fig. IV *a* and *b*). Behind this region the cross-section becomes elliptical, and then almost circular towards the tail, which again is usually flattened and sometimes tapers rather abruptly to a point (Fig. IV *c*). When the gregarine is alive, the body is thrown into S-shaped curves. It attaches itself to the surface of the epithelium by an anterior, more hyaline, knob-like portion which has gradually become marked off from the rest of the body by a slight constriction. In the living condition, a delicate, beak-like process extends beyond the knob and lies in a depression between two cells or in a cell

surface; but it does not actually penetrate a cell. This terminal process becomes contracted on fixation by reagents; we seldom find it in preserved specimens. The knob contains about four refringent, rod-shaped bodies, lying just below the pellicle; sometimes a vacuole can be seen in the middle of the knob. The little rods are the persistent siderophilous striations of the merozoite stage; they still stain densely with iron haematoxylin.

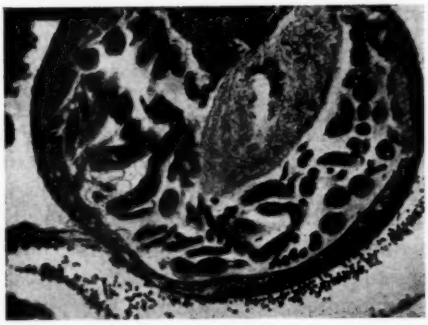
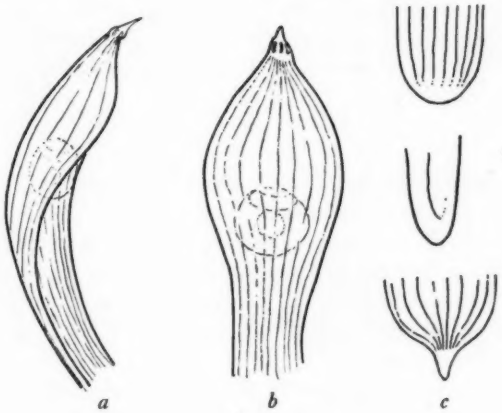


Fig. III. Transverse section of the gut of *Potamilla reniformis* showing large numbers of trophozoites of *Meroselenidium keilini*. × 260. (D. A. Kempson, phot.)



IV

Fig. IV. *Meroselenidium keilini*. Freehand drawings of living trophozoites. *a* and *b* show the anterior end in side view and face on. *c* shows the appearance of the posterior end in different specimens. × 1000.

From this head region a large number of shallow grooves run back along the pellicle; there are thirty to forty such grooves, and between them a corresponding number of ridges; these ridges tend to be narrower and less convex on the flattened surface of the animal (Fig. 15), and here they stain more

darkly; in well-differentiated specimens each shows as a double black line with a more faintly staining area between.

The spherical nucleus lies in the bravest region of the body (Figs. 14 and 16). There is a well-defined nuclear membrane, from which, in preserved material, the contents always shrink away slightly, however careful has been the fixation. Some of the chromatic substance is distributed in irregular patchy clouds in the nuclear sap; there is a tendency for some of it to lie near the periphery, attached to the membrane by strands, and for a larger patch to occupy the centre. Usually within this central patch, but sometimes further out, is a larger, densely staining granule. At the anterior pole of the nucleus there is always a highly refringent blob of chromatic substance, which stains dense black with iron-haematoxylin and red with Mann's methyl-blue-eosin. It seems to develop from the large karyosome noticeable while the parasite is still intracellular. When the gregarine becomes free, this mass moves to the anterior pole of the nucleus (Fig. 13), assumes a cap-like shape (Fig. 17) and becomes increasingly hollowed out on its posterior face until it has the form of a ring (Figs. 18, 16 and 14). The ring is very often thicker at one side than the other, and it may be incomplete at one point. Exceptionally it may happen that the ring develops while the parasite is still small and intracellular (Fig. 11). The presence of this body is very characteristic of *Meroselenidium keilini*, and, even if there were no other means of distinguishing its free trophozoites from those of its fellow gregarine, the nuclear structure would be a sure clue.

The cytoplasm of the large trophozoites is often parasitised by minute organisms similar to those noticed in the bodies of selenidia by Ray and other protozoologists (Fig. 19).

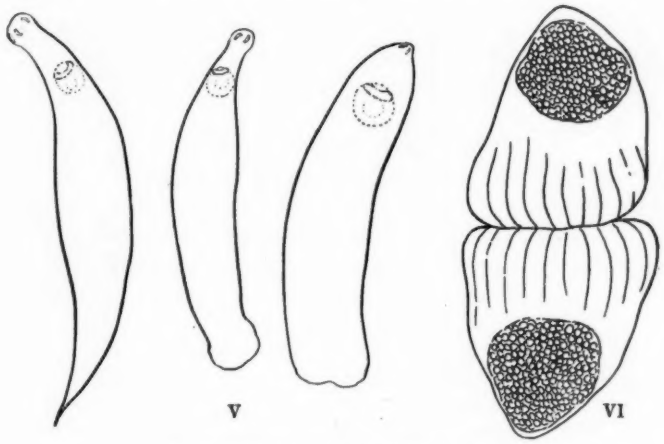
(3) *Sporogony.*

Since there is no indication that the gregarines in the gut lumen reproduce asexually, it would seem reasonable to suppose that they are all potential sporonts. This may be so; but we have observed that association generally takes place further forward in the intestine than the place where the free trophozoites are most abundant, and we have also noticed that those obviously preparing for association are almost always shorter, though not much broader, than the full-grown forms.

The pre-associates are usually 90–100μ long and 18–22μ broad, and the post-nuclear portion is relatively short and blunt (Fig. V). Such short, blunt-tailed gregarines move sluggishly, and the shortening and thickening continues. Finally they associate in pairs by their posterior ends, and the outline of their juxtaposed bodies takes the form of an ellipse measuring about $85 \times 40\mu$; one of the partners is sometimes larger than the other.

For a time the anterior ends of the gametocytes project from the poles of this mass; but they are gradually withdrawn. The characteristic pellicular striations are still for a time visible. When association takes place, but sometimes before this happens, the nuclei of the gametocytes become relatively very

large, and seem to be filled with refringent spherules (Fig. VI); but gradually the cytoplasm becomes so dense that it is impossible to observe in the living organisms what happens next. Stained sections indicate, however, that the gamete nuclei are formed within the enlarged nuclear area of the gametocyte in the peculiar manner first observed by Caullery and Mesnil (1899 and 1900) for species of *Selenidium* in *Scolelepis fuliginosa* and in *Spio martinensis* and described again by Ray (1930 *a*). Fig. 20 shows the anterior end of an unattached gregarine preparing for association, in which the nucleus is filled with minute mitotic figures. In Fig. 21 the daughter nuclei so formed are clearly visible, while the membrane of the parent nucleus still persists. The nuclear membrane fades and the small nuclei wander out into the cytoplasm (Fig. 22), sometimes accompanied by a cloud of dark-staining chromatic dust, which seems then to be absorbed. In Fig. 22 it can be seen that a minute siderophilous granule lies at one pole of many of the nuclei.



Figs. V and VI. *Meroselenidium keilini.*

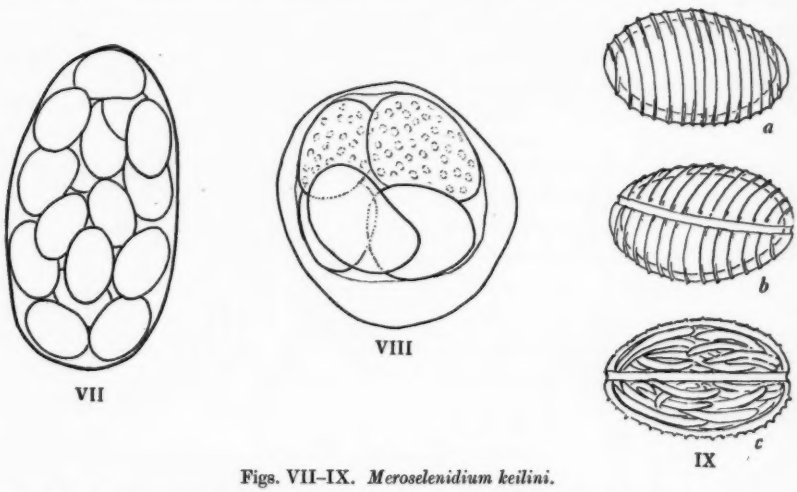
Fig. V. Trophozoites shortening previous to association. × 1000.
Fig. VI. Paired gametocytes, the enlarged nuclei of which are filled with minute spherules. × 900.

For the study of further stages we have adopted the method described on p. 145, and after repeated failures we secured gametocysts on seven different occasions. The naked worms, which have been left in filtered sea water overnight, secrete a sticky mucus about their bodies, and to this the faecal matter adheres, together with some ejected parasites[1]. Pieces of this substance were placed in a moist chamber and examined from hour to hour.

Though later we searched the mucus carefully at shorter intervals after the segregation of the experimental worms, we never found cysts containing

[1] We are not certain whether the gametocytes escape from the anus of the worm or from its mouth.

gametes. It may be objected, we feel, that only where the gametes have been seen and measured and where their union has been observed to give such spores as the product we shall describe, can one be *certain* that the end-result is really connected with the paired gametocytes of a particular species. We would point out, however, that in general appearance and in the absence of any residual mass, spore-containing gametocysts agree very well with what Ray records of the gametocysts of *Selenidium spionis* and *S. foliatum*, and that their dimensions are just what we should expect from those of the paired associates of *Meroselenidium keilini*. Moreover, by this time we are only too familiar with all the usual intestinal contents of Potamilla and with the organic materials



Figs. VII–IX. *Meroselenidium keilini.*

Fig. VII. Gametocyst with sporocysts. Freehand drawing from living specimen. Note the absence of any residual mass. × 500.

Fig. VIII. Portion of a gametocyst containing five sporoblasts. Freehand drawing from a specimen treated with Lugol's solution. Note the numerous nuclei. × 1000.

Fig. IX. Sporocysts. Freehand drawing from living material. *a* shows the transverse ridges, *b* also shows the longitudinal groove, *c* the numerous sporozoites. × 1400.

that may adhere to its body; and we have taken every precaution possible, by washing and by filtering, to exclude contamination of the water by foreign bodies.

What we find then are groups of relatively large sporocysts, each group being elliptical in outline and measuring about $70 \times 55\mu$ on an average (Fig. VII). There are about twenty sporocysts in a group. The envelope containing them is very delicate and is easily ruptured; then the sporocysts lie about in smaller groups of five to ten each (Fig. VIII). It is remarkable that the number of sporocysts is always small in relation to the number of gamete nuclei in the paired gametocytes. There is never any residual body.

The sporocysts measure about $26–28 \times 14–16\mu$. The transparent envelope

is well defined; and under higher magnification shows the outline broken by minute prickles, which careful focussing reveals as optical sections of delicate ridges running crosswise over the spore's surface (Fig. IX *a*). The sporocyst appears to be bivalve; at any rate, as it tilts over, a clear groove can be seen running round it lengthwise (Fig. IX *b*). But it is the protoplasmic contents that offer the really unusual feature of these spores. When they are living it is impossible to see any internal detail; but when treated with Lugol's solution, numerous minute nuclei appear all over the cytoplasm of a sporoblast (Fig. VIII): this is confirmed by study of the stained sections. Fig. 23, drawn from a section, shows a sporocyst containing many ring-like nuclei. The final product is a large number of minute sporozoites packed closely together within the sporocyst (Fig. IX *c*); there seems to be no residual body in the spore. We have seen the sporozoites only in material treated with Lugol: we have found them in none of our whole mounts or sections.

The definition of the genus *Meroselenidium*, then, should run as follows:

Schizogregarine with intracellular schizogony initiated by the formation of smaller masses which give rise to the merozoites. Sporocysts polyzoic. Type species M. keilini, *in the gut of the polychaete worm* Potamilla reniformis (O.F.M.) *at Plymouth.*

B. *Selenidium potamillae* n.sp.

Unlike the last species, this gregarine is practically confined to the anterior half of the gut of *Potamilla*. We found it in about 50 per cent. of the worms examined. To some extent the regions infected by the two selenidia overlap, but in a general way it is true that they divide the gut between them, each confining itself to its own particular stretch of epithelium.

(1) *Schizogony.*

We know less about the asexual reproduction in this gregarine than in the last, and we have not been able to distinguish with certainty between undivided schizonts and those forms which, developing from merozoites, will later become free in the gut lumen.

The dividing schizonts are intracellular, and, just as in *Meroselenidium keilini*, commonly occur in pairs. At first they are elliptical in outline and measure anything from $11 \times 7\mu$ to $18 \times 12\mu$. The pellicle is extremely thin and the cytoplasm much vacuolated and fragile looking during the early stages of nuclear division; later it becomes more condensed and the body tends to assume a sub-spherical form. Fig. 24 shows a schizont in which the nucleus is dividing for the first time; in Fig. 25 other nuclei are forming; Fig. 26 is of a smaller schizont filled with nuclear spindles; Fig. 27 shows the resting nuclei of the future merozoites—these nuclei are ring-like, and minute siderophilous granules lie near them in the cytoplasm. There is nothing here in the nature of cytomere formation. At a later stage (Fig. 28) these small granules are no longer apparent, and their place seems to be taken by dark-staining bodies of approximately the same size as the now very pale nuclei they accompany: it

seems probable that the small granules (of whose origin, however, we know nothing) give rise by growth to the larger masses. Later still, the merozoites are differentiated as tiny, sickle-shaped gregarinulae, only 2·5μ long, closely applied to the surface of the sub-spherical schizont (Fig. 29). They break away from this, leaving a large residual mass in the centre. The nucleus of each merozoite stains very faintly and looks like a mere vacuole at the posterior end. The siderophilous body has elongated and lies in the anterior half, where it forms a dark band along the more convex surface (Fig. 30). The merozoites wander out into the adjacent cells (Fig. 29). As the large intracellular forms with which (on the analogy of *Meroselenidium keilini*) it is reasonable to connect their development, are without any characteristic anterior striate differentiation, it is much more difficult in this species to follow out the growth stages. But patches of the epithelium are crammed with intracellular gregarines, varying in size from 8×6 to $40 \times 28\mu$, and the density of the infection in these patches strongly suggests that the majority of the parasites have developed from merozoites which have wandered but a short way from the foci provided by a few schizonts.

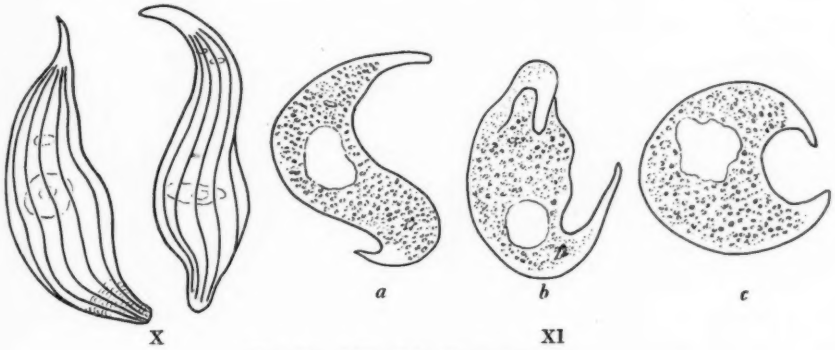
Figs. 31, 32 and 33 are drawn from sections through infected epithelium. The gregarine is more pointed at one end than the other: transverse sections show that it is flattened on one surface. The pellicle is ridged. In contrast with *Meroselenidium keilini*, the number of these longitudinal ridges is small, never more than eighteen: those on the flattened side are usually narrower and flatter than those on the convex. At this stage the imprisoned gregarine frequently lies curved round the nucleus of the host cell (Fig. 32), which may be hypertrophied (Fig. 33). Its own nucleus, which lies midway in its body, has an outline varying from circular to transversely elliptical, according to the degree of compression; it contains one large and frequently also one very small, densely-staining karyosome—these lie in a cloud of paler chromatin granules. The cytoplasm usually stains much more deeply and patchily than does that of *Meroselenidium*; it contains some siderophilous granules of various shapes and sizes; groups of these frequently lie fore and aft of the nucleus, but there is never the constant arrangement of siderophilous bodies at the anterior end of this gregarine such as we find in the trophozoite of *Meroselenidium*.

(2) *The adult trophozoite.*

This *Selenidium* seems to grow to approximately full size before it emerges into the gut lumen. Occasionally we have found individuals measuring $63 \times 20\mu$, but $40 \times 15\mu$ is the common size. Once free, it stretches out, and the difference between anterior and posterior ends becomes more marked; the former is drawn out into a clear, beak-like point (Fig. X) which serves probably for attachment to crypts between the epithelial cells. There is little movement of translation; the animal writhes about and throws itself this way and that, while waves of contraction may temporarily deform the almost leaf-like body (Fig. XI). We have never before seen any species of *Selenidium*

perform these "euglenoid" movements: they are very different from the pendular swaying and nematode-like wriggling so characteristic of the great majority of selenidia. The superficial striations on the pellicle are clearly visible, especially towards the posterior end, where transverse wrinkles may also appear.

The endoplasm is densely granular, and it is impossible to see any other internal structure than the nucleus, which shows as a clear patch about the middle of the body; its outline is constantly deformed by the animal's contortions. The nucleus appears much as it does while the parasite is intracellular, except that the whole stains more uniformly and the large karyosome almost always lies towards one side; the absence of the characteristic siderophilous ring makes it easy to distinguish the trophozoites of *Selenidium potamillae* from those of its companion in the gut.



Figs. X and XI. *Selenidium potamillae.*

Fig. X. Freehand drawings of the living trophozoite, showing longitudinal striations. × 630.
Fig. XI. Freehand drawings of the living trophozoite to show successive positions (*a*, *b* and *c*) assumed by one individual. Striations omitted. × 630.

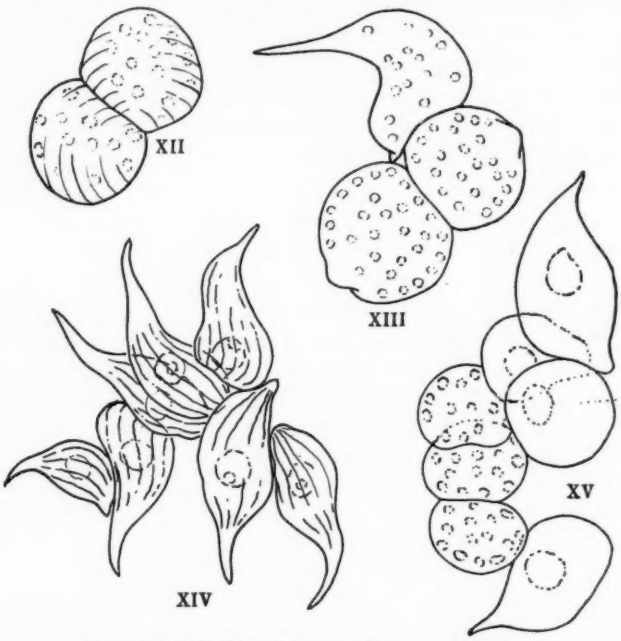
(3) *Sporogony.*

There seems no good reason to doubt that such trophozoites are all potential gametocytes. They seem to become sticky on the surface and adhere by their posterior ends in pairs. For some time the actively moving, beak-like anterior ends project; but as the bodies thicken and shorten these are withdrawn (Fig. XII). The pellicular striations can still be seen; and their small number, together with the much smaller size of the couple, serves to distinguish the paired gametocytes of *Selenidium potamillae* from those of *Meroselenidium keilini*, along with which they sometimes occur.

But very commonly more than two parasites combine. A group of three is frequent (Fig. XIII), and we have seen groups of five, six, and even seven (Figs. XIV and XV). The members of such a group are not necessarily all at the same stage; some may be rounded off while the others are still elongate and

active, and the contractions of these cause the mass of adherent bodies to turn slowly about. Stained sections of infected gut show clearly that this curious tendency to group, which we have seen in no other associating *Selenidium*, is not just an abnormality induced by concentration of the medium while the living gregarines are under observation.

Nuclear changes usually begin after association, and often before the subspherical form has been assumed (Fig. XIII). The gamete nuclei are at first



Figs. XII–XV. *Selenidium potamillae.* × 600 approx.

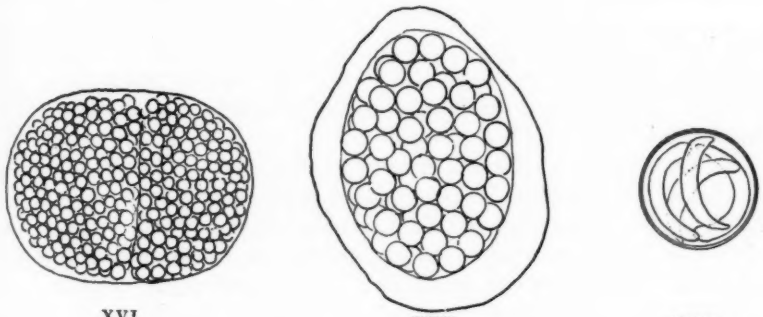
Fig. XII. Associated gametocytes.
Fig. XIII. Three gametocytes in one group.
Fig. XIV. Seven individuals attached to one another by their posterior ends.
Fig. XV. Seven individuals in a group; three of them have rounded off and contain numerous nuclei.

(All drawn freehand from living material.)

crowded together in what appears to be the original nuclear area of the associate, and then they wander to the periphery in a cloud of chromatic substance (Fig. 34).

The paired gametocytes do not develop further in the body of the host, and we adopted the same methods as for *Meroselenidium* to obtain the spores (see p. 145). We have not seen the escape of the sexual forms from the gut, but the fact that they usually lie far forward in the lumen suggests that they may normally be expelled by the mouth and not by the anus; tube-dwelling worms

like *Potamilla* withdraw suddenly into shelter when disturbed, and at such moments matter lying free in the fore part of the gut might easily be driven out. Ripe gametocysts may be found in the mucus around a worm twelve hours after the animal has been placed in filtered sea water kept under aeration. They are of a size consistent with that of the associating gregarines, *i.e.* little more than half that of the gametocysts of *Meroselenidium*. The gametocyst of *Selenidium potamillae* is occasionally spherical, but more frequently is elliptical in outline; an endocyst enwraps the sporocysts closely, while a thicker exocyst lies some way further out (Fig. XVII). As was to be expected, we constantly found the gametocysts of this species in groups, and the members of a group might differ not a little in size: $45 \times 33\mu$ were the dimensions of the largest we saw. This size difference is explicable on the assumption that sometimes more than two associates may contribute to the contents of one gametocyst.



Figs. XVI–XVIII. *Selenidium potamillae.*

Fig. XVI. Gametocyst containing gametes. × 660 approx.
Fig. XVII. Gametocyst containing spores. × 500 approx.
Fig. XVIII. Sporocyst containing four sporozoites. × 1500.
(All drawn freehand from living material.)

In two living gametocysts (Fig. XI) only have we seen the gametes; these were all alike, spherical, and 4 to 5μ in diameter. The copulae (sporoblasts) are also nearly spherical in this species of *Selenidium*, and are from 7·5 to 8·5μ in diameter. They are numerous, there being more than fifty in a gametocyst. No residual mass is left. The ripe sporocyst has a smooth surface. Its contents, when treated with Lugol's solution, usually show four nuclei, but occasionally there are more. We only once found sporocysts containing the four sporozoites (Fig. XVIII); the sporozoites were about 8μ long, and very slender. We never found gametes or sporozoites in our stained sections. Fig. 35 shows a section of a gametocyst containing what we consider to be uninucleate sporoblasts. Fig. 36 is a portion of another gametocyst, containing four-nucleate sporocysts; each has a rather large vacuole. To find only four nuclei is the rule; but sporocysts with six, eight, and even with ten nuclei occur. These cysts are

rather larger than the normal; one containing eight nuclei is shown in Fig. 37. In the same gametocyst with the uninucleate sporoblasts (Fig. 35) we observed one binucleate body about double the size of the others (Fig. 38). At first we thought that this was a stage in fusion of two gametes; but the dimensions are too large, and we consider it more probable that occasionally two copulae fuse and proceed to divide within a common sporocyst; this would help to account for the abnormal number of nuclei observed in the larger sporocysts here and there.

Diagnosis of the species:

Selenidium potamillae *shows intracellular schizogony. The trophozoites are "euglenoid." The smooth-walled sporocysts contain four sporozoites as a rule, but may have as many as ten. Habitat: the gut of* Potamilla reniformis (O.F.M.) *at Plymouth.*

IV. General considerations.

It is clear that with a worm of such gregarious habit as is *Potamilla reniformis*, infection must spread easily, for the released gametocysts or sporocysts of the two parasites would readily be whirled into the mouth along with the minute débris on which the creature feeds.

We have not yet succeeded in getting spores to hatch nor have we observed the entry of the sporozoites into the epithelium of the gut nor the early stages of their growth there. But with the exception of these details we have now before us in their right sequence the essential features in the whole life cycle of the two schizogregarines that parasitise *Potamilla reniformis*.

The schizogregarines have attracted the attention of those protozoologists who enjoy speculation concerning possible relationships between the various groups of Sporozoa. The phase of asexual multiplication is reminiscent of what one finds in the *Coccidiomorpha*. Certain authorities regard this as a primitive feature: others, such as Keilin (1923) think that the schizogony in gregarines is a character secondarily acquired and that the schizogregarines have been derived from various eugregarine stocks. Naville (1931) considers that the eugregarines and schizogregarines have had a common origin from some very ancient stock which showed features in its life cycle common also to that of the *Coccidiomorpha*. Most of the schizogregarines in which the whole life cycle has been fully worked out are coelomic parasites and so presumably less likely than gut parasites to show primitive features. Forms like *Ophryocystis* and *Schizocystis*, which occur in the lumen, never show the intracellular habit characteristic of coccidians. *Selenidium*, then, seemed to us a promising subject for investigation, since it is a gut parasite, but, to some degree at any rate, committed to intracellular life: of the two parasites described in this paper, one, *Selenidium potamillae*, is intracellular until the approach of the associating period.

We cannot pretend that we have discovered any facts that would determine us to vote in favour of Naville's theory rather than those of earlier observers;

but there are one or two points in the life history of schizogregarines to which we would like to draw attention before we sum up. And the first is that, for a parallel to the peculiar method of merozoite formation in *Meroselenidium keilini*, we have to go to the coccidia. Then, while it is true that the majority of the known schizogregarines yield eight sporozoites per spore, Spirocystis is said to have but one, "*Selenidium*" apparently gives a variable number—four in *S. echinatum*, *S. spionis*, *S. mesnili*[1] and *S. potamillae* (though hyperzoic spores also occur in this last species) and eight in *S. foliatum*—while *Meroselenidium* is polyzoic. Now typical gregarines always have eight sporozoites; and the variability in the sporozoite number within the group of schizogregarines makes one think again of the coccidians. Moreover, it would seem that this variability may be expressed within the spores of one species. Steopoe (1928) mentions two categories of spores of different dimensions in the schizogregarine *Syncystis mirabilis*; and, as we have just shown, the spores are sometimes of different sizes in *Selenidium potamillae*, the larger ones containing more than the normal number of nuclei. This state of affairs is again paralleled among the coccidia: *Aggregata eberthi* has normally three sporozoites, but Dobell (1925) describes larger hyperzoic spores containing four, six, eight, or even as many as sixteen.

On the other hand, no schizogregarine shows fertilisation phenomena comparable with those in the coccidian. Except that they rarely show a residual body in the gametocyst (*Schizocystis* seems to be an exception), the sporogony in schizogregarines is in all essentials like that of eugregarines. The majority form isogametes, and, if *Selenidium potamillae* is typical of the rest of its group, this is true also of "*Selenidium*."

V. SUMMARY.

1. Two new schizogregarines are described from the gut of the polychaete worm *Potamilla reniformis* (O.F.M.). One of these is meanwhile retained in the genus *Selenidium* Giard as *S. potamillae* n.sp. For the other a new genus, *Meroselenidium*, is proposed, of which *M. keilini* is the type species. The life cycle has been followed in both gregarines.

2. The schizogony of *Meroselenidium* is characterised by the formation of cytomeres preliminary to the liberation of merozoites. In *Selenidium potamillae* the schizogony is more like that in *S. mesnili* and *S. caulleryi*, though there is a considerable residual body.

3. The gametocytes become attached by their posterior ends. In *S. potamillae* there is often a multiple association. The gamete nuclei are formed within the nuclear area of the gametocytes. Further development takes place in sea water. Isogametes have been observed in *S. potamillae*.

4. The gametocyst of *Meroselenidium keilini* is elliptical in outline and measures about $70 \times 55\mu$. It contains some twenty relatively large spores, $27 \times 15\mu$, the walls of which are transversely ridged; the spores are polyzoic.

[1] See foot-note on p. 144.

The gametocyst of *S. potamillae* is of much the same shape, but only about half the size. The sporocysts are very numerous, spherical, smooth-walled, and measure about 8·5μ in diameter. In neither species does the gametocyst contain any residual mass.

We take this opportunity of thanking the High Commissioner of India and the Delegacy of King's College for grants-in-aid received by one of us (H. N. R.) during the course of these investigations.

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EXPLANATION OF PLATES XIII TO XVII.

All drawings were made with the aid of a camera lucida, and almost all from sections of worms fixed with alcoholic Bouin and stained with Heidenhain's iron-haematoxylin counterstained with chromotrop 2R. Figs. 14 and 34 are from smears of gut contents, and Figs. 35–38 from sections of gametocysts fixed with Carnoy's fluid and stained with Delafield's haematoxylin and chromotrop 2R.

PLATE XIII.

Meroselenidium keilini n.gen., n.sp.

Fig. 1. Half-grown schizont in the gut epithelium. Note the large nucleus with its two karyosomes. × 1000.

Fig. 2. Larger schizont lying beneath the gut epithelium. × 1000.

Fig. 3. Two full-grown schizonts lying side by side. They have assumed a pear shape, the narrow end directed towards the gut lumen. The pellicle shows striations. In the section the nucleus shows in only one of the pair: note its relatively small size and the faint staining of the larger karyosome. × 1000.

Fig. 4. Surface view of one end of a large schizont, showing longitudinal striations of the pellicle and, in the grooves between some of these ridges, transversely running lines. × 1000.

Fig. 5. Sub-epithelial schizont showing three small nuclei and several nuclear spindles. (Reconstructed from three successive sections.) × 1000.

Fig. 6. Schizont divided into "cytomeres"; each is studded with small dense nuclei. Note the persistent, folded pellicle. × 1000.

PLATE XIV.

Meroselenidium keilini n.gen., n.sp.

Fig. 7. The schizont liberating its merozoites. Note the residual body in the centre of each mass. × 1000.

Fig. 8. Portion of a cytomere with its ripe merozoites. Note the dark-staining lines at their distal ends and the position of the ring-like nuclei. × 1400.

Fig. 9. Merozoites which have migrated from a schizont into other epithelial cells. Note their blunt anterior ends, the persistent siderophilous lines, and the position of the nucleus midway in the body and containing three dark-staining granules. × 1400.

Fig. 10. Two somewhat larger individuals, showing the same characteristics as in the last figure. × 1400.

Fig. 11. Considerably larger and more elongated individual. The nucleus here is approximately of the type assumed in the adult. × 1400.

Fig. 12. A larger gregarine, but still intracellular. The nucleus here is more as it usually appears at this imprisoned stage. × 1000.

Fig. 13. A small gregarine from the lumen of the gut. Probably this has developed from one of a group of merozoites liberated directly into the lumen by bursting of the host cell round the schizont. (Cilia of epithelium omitted.) × 1000.

Fig. 14. Full-grown free gregarine in the gut lumen. Note the pellicular striations, the knob-like "head" with rods and protoplasmic beak, and the nucleus with the chromatic ring. (From a smear.) × 1000.

Fig. 15. Transverse section of a full-grown gregarine, to show the large number of pellicular ridges: those on the flattened surface are narrower and retain the stain better than those on the convex surface. × 1000.

PLATE XV.

Meroselenidium keilini, n.gen., n.sp.

Fig. 16. Section of the anterior end of an attached adult gregarine. (The protoplasmic beak is withdrawn.) The nucleus shows the features characteristic of this stage. (Cilia of epithelium omitted.) × 1000.

Fig. 17. Nucleus of a rather younger specimen, showing the hollowed out anterior cap of chromatic material not yet developed into a ring. × 1400.

Fig. 18. Oblique section in the region of the nucleus showing the thinning out of the anterior chromatic cap to form a ring. × 1400.

Fig. 19. Portion of the posterior end of a gregarine containing multiplying parasites. × 1000.

Fig. 20. Longitudinal section through the anterior end of a gregarine about to enter on the sexual phase. The nuclear membrane is fading and the interior of the nucleus contains numerous small spindles. × 1000.

Fig. 21. Oblique section of a thickened, shortened gregarine, showing the enlarged nucleus with many small nuclei within the membrane. × 1000.

Fig. 22. Section through a pair of gametocytes attached by their posterior ends. Two of the anterior siderophilous bodies still show in one of them. Note the numerous nuclei now scattered in the cytoplasm. × 1000.

Fig. 23. Sporocyst. Note the numerous nuclei. × 1400.

PLATE XVI.

Selenidium potamillae n.sp.

Fig. 24. Intracellular schizont showing the first division of the nucleus. × 2000.

Fig. 25. Schizont showing further nuclear division. × 2000.

Fig. 26. Small schizont containing many nuclear spindles in anaphase. × 2000.

Fig. 27. Sub-spherical schizont with numerous resting nuclei on the surface: accompanying them are siderophilous granules. × 2000.

Fig. 28. One of a pair of schizonts with pale ring-like nuclei, each accompanied by a siderophilous mass of approximately the same size. × 2000.

Fig. 29. One of a pair of ripe schizonts liberating its merozoites into the surrounding tissue. Note the residual mass underlying them. × 2000.

Fig. 30. Three merozoites greatly magnified. Note the siderophilous line along one side of each and the terminal vacuole-like nucleus. × 5000.

Fig. 31. Section through the epithelium showing full-grown gregarines, still intracellular, cut through at various levels. Note the relatively small number of pellicular striations in this species. × 1500.

PLATE XVII.

Selenidium potamillae, n.sp.

Fig. 32. Full-grown intracellular gregarine cut through its length. Note the siderophilous granules in the cytoplasm. (Cilia of cells omitted.) × 1500.

Fig. 33. Sections of intracellular gregarines. Note the hypertrophied nucleus of the host cell. (Cilia omitted.) × 1500.

Fig. 34. Paired gametocytes containing numerous nuclei. (From a smear.) × 1450.

Fig. 35. Section through a gametocyst containing numerous uninucleate sporoblasts. × 1450.

Fig. 36. Portion of a sectioned gametocyst containing sporocysts each with four nuclei. × 1450.

Fig. 37. A large sporocyst containing eight nuclei. × 1450.

Fig. 38. Binucleate sporoblast. × 2900.

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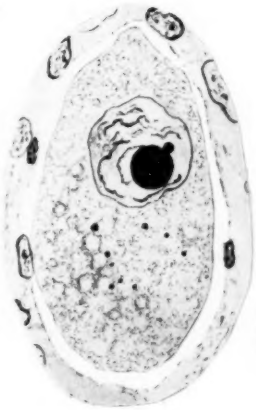

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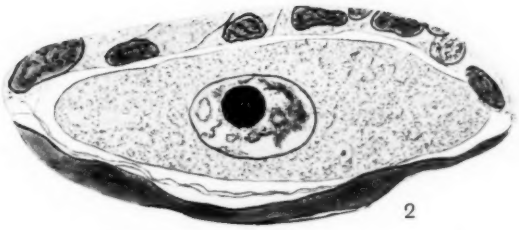

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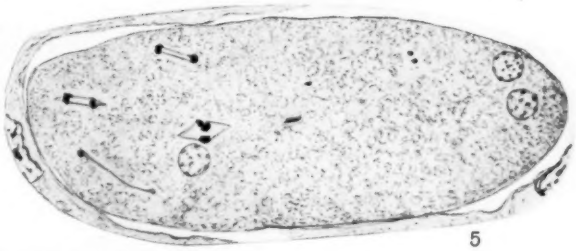

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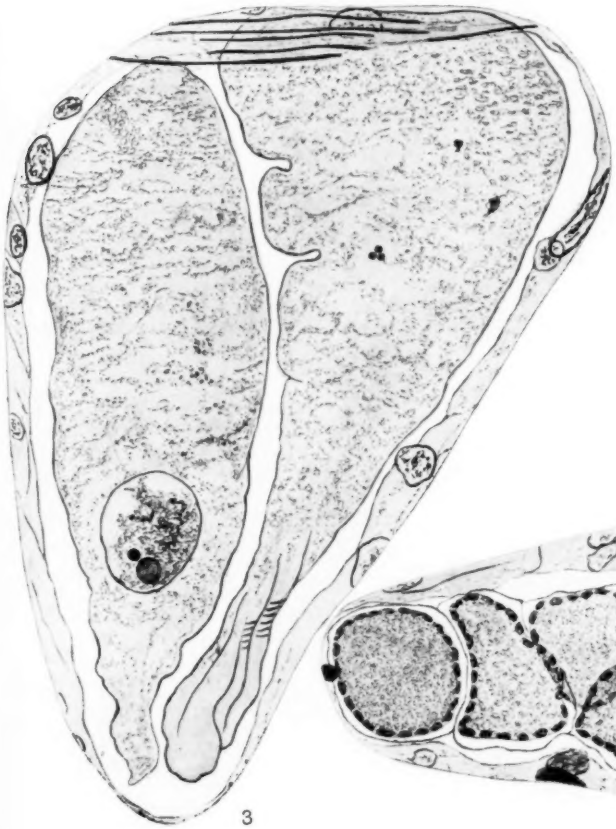

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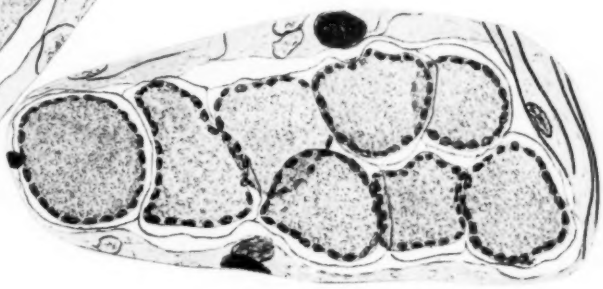

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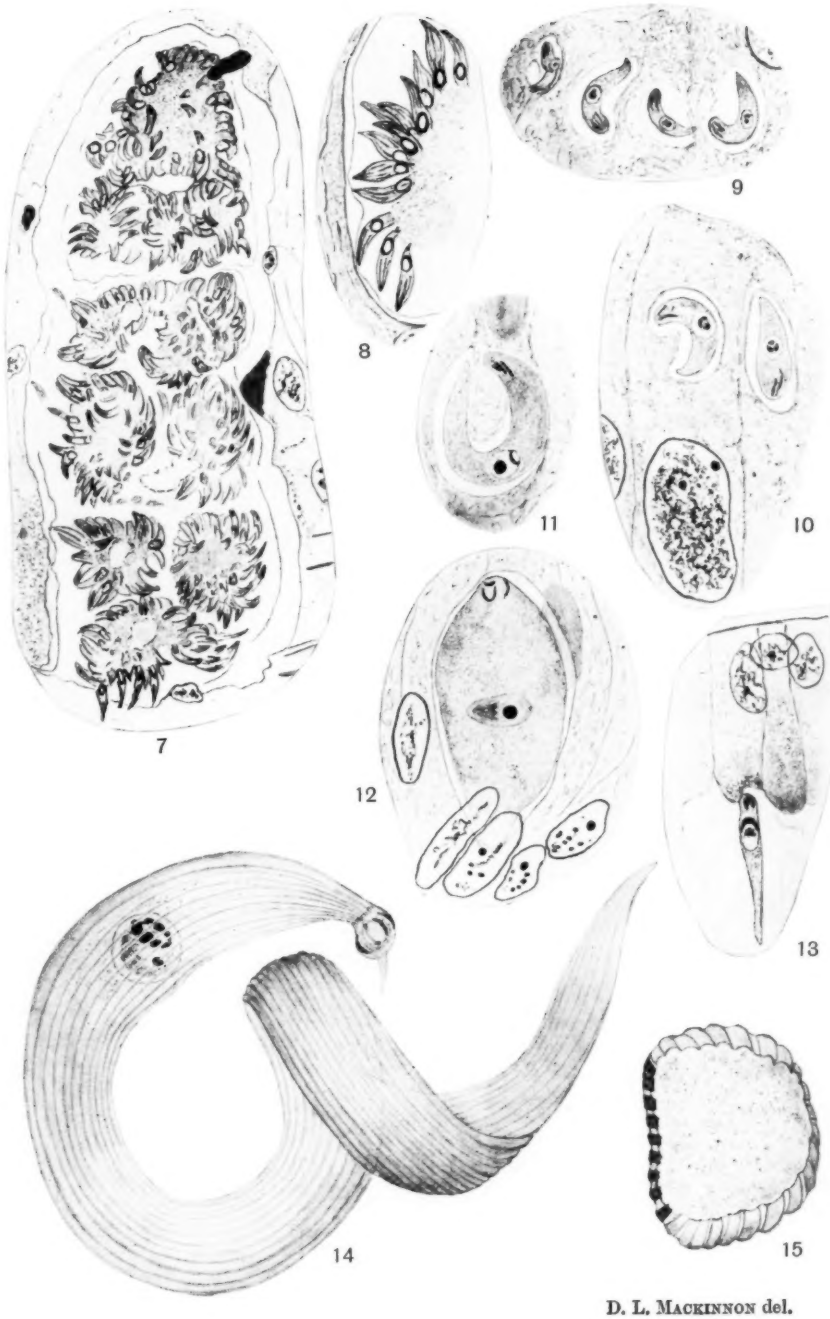

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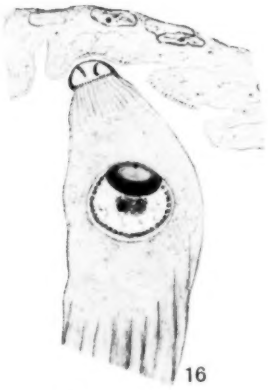

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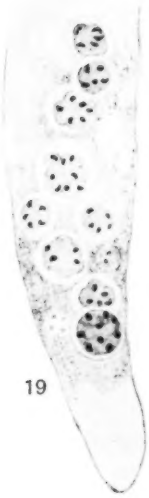

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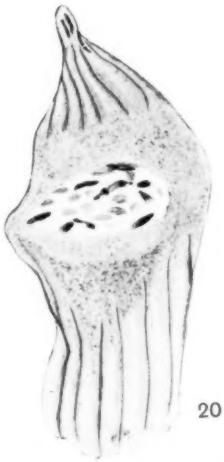

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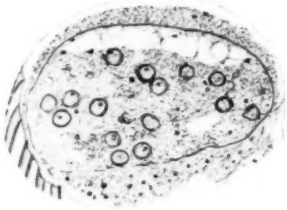

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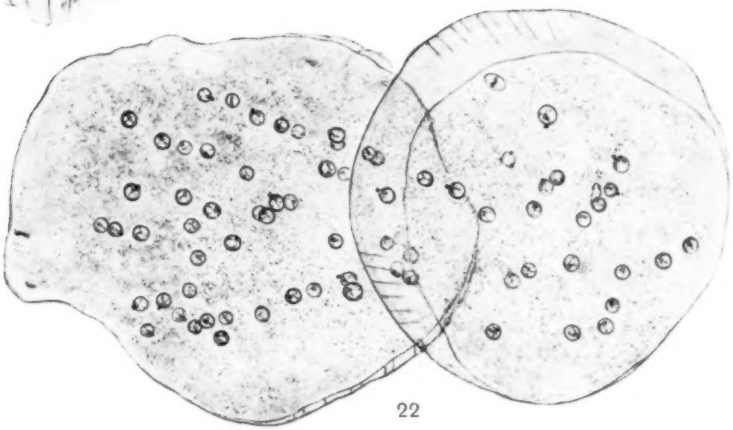

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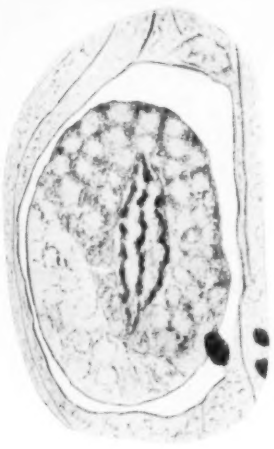

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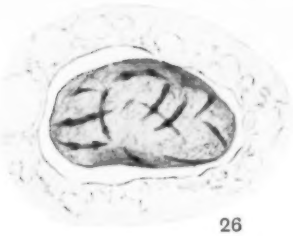

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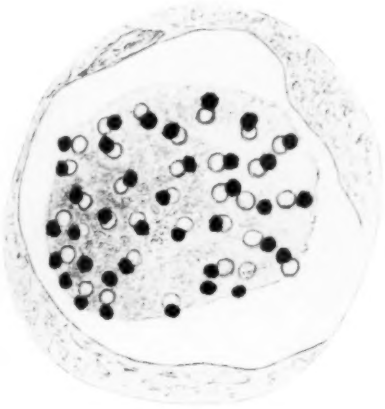

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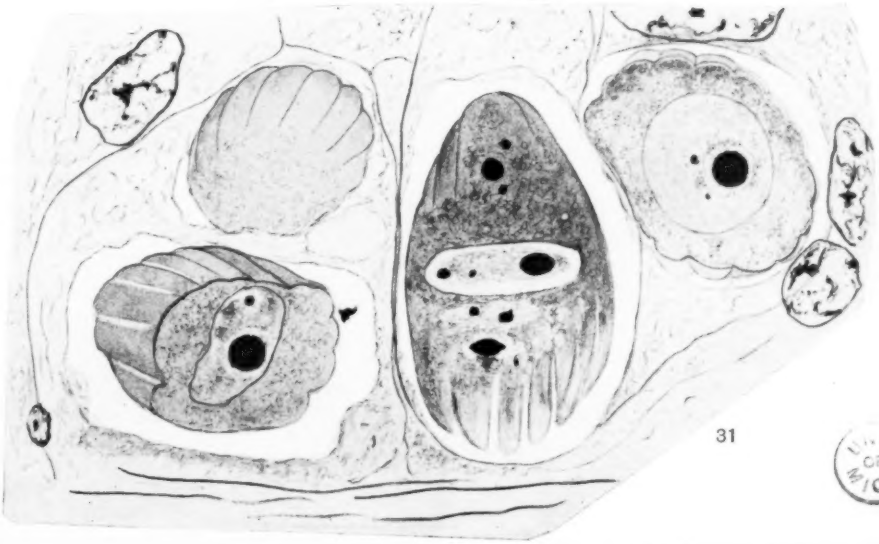

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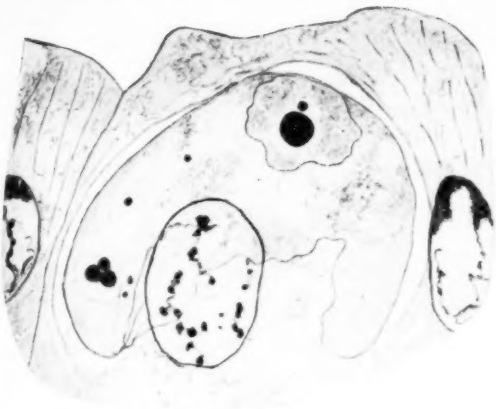

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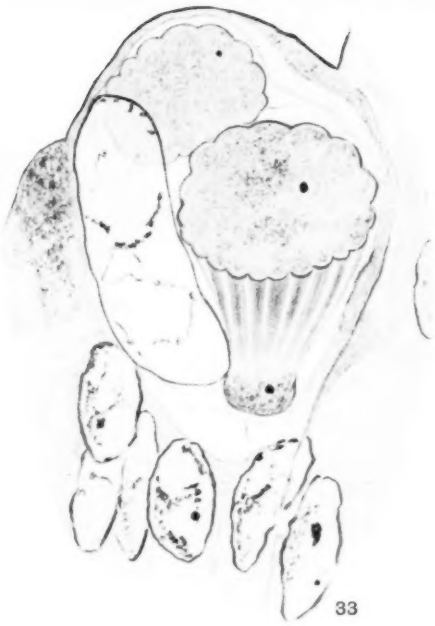

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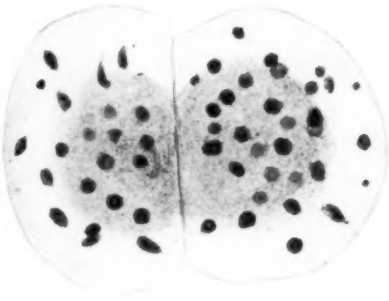

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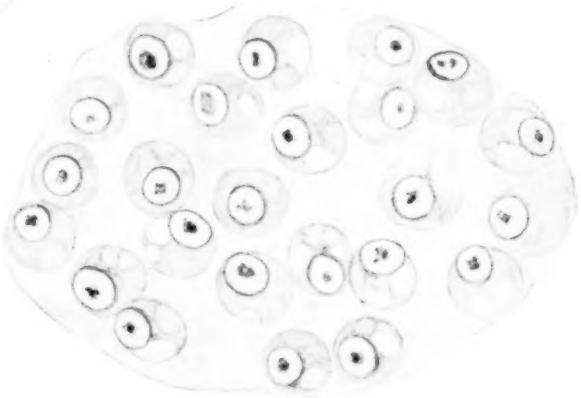

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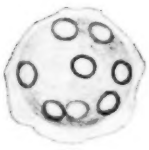

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THE MORPHOLOGY AND DIVISION OF *HEXAMITA GIGAS* N.SP. (FLAGELLATA)[1].

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(With Plate XVIII, containing Figs. 1–13, and 10 Figs in the Text.)

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I. Introduction.

A previous paper (Bishop, 1932) dealt with the cultivation of a *Hexamita* sp. found in the horse-leech (*Haemopis sanguisugae* = *Aulastomum gulo*). The present paper deals with the morphology and method of division of the *Hexamita*[2] above referred to, which I now name *H. gigas* n.sp. Dobell (1909) gave a detailed history of the nomenclature of the genus, and Wenyon (1926) has dealt with the same subject and given a list of the then known species and their hosts. Two species have since been described: *H. pulcher*, by Becker (1926), from the caecum of the ground squirrel *Citellus tridecemlineatus*, and *Octomitus* (= *Hexamita*) *pitheci* described and figured by da Cunha and Muniz (1928) from the intestine of *Macacus rhesus*. Apart from the two species (*Hexamita nodulosa* and *H. inflata*) described by Dujardin (1841) from stagnant water, and in which he saw but six flagella, a single species only has been described hitherto from a non-vertebrate host. This species, *Octomitus* (= *Hexamita*) *periplanetae*, a small flagellate 5–8 μ in length, from the gut of the cockroach, was described by Bělař (1916); a similar flagellate from the gut of *Blatta orientalis* has been found by me.

[1] A study begun during my tenure of a Beit Memorial Fellowship and completed since I became a Yarrow Fellow.

[2] Since *Hexamita* Dujardin 1841 has priority, the use of *Hexamitus* for this genus by some subsequent authors is unjustified. The generic name *Hexamita* is used throughout this paper.

II. Material and methods.

Mixed cultures of *Hexamita gigas* with *Entamoeba aulastomi* were obtained from the gut of a horse-leech dissected on 19. vi. 31. As previously stated (Bishop, 1932), in September of that year, Mr Clifford Dobell, F.R.S., separated the strain of *Hexamita* from the entamoebae. Since then it has grown luxuriantly until now (7. xii. 32). Two media have been used; an inspissated horse-serum slope covered with inactivated horse-serum diluted 1 : 10 in 0·5 NaCl solution, or this liquid medium alone. To this latter medium a little solid sterile rice starch was added before inoculation. Cultures grown on the part-liquid part-solid medium required subcultivating every 7 days when grown at room temperature, and every 10–14 days when grown at + 4° C., whereas those grown on the wholly liquid medium, though never so rich, lived 10–20 days at room temperature and a month to 6 weeks at + 4° C.

Smears to be stained with aqueous iron haematoxylin were fixed with either Bouin's aqueous fixative containing acetic acid, or with Schaudinn's solution with 2·5 per cent. glacial acetic acid. The films were hardened in 90 per cent. alcohol for at least 48 hours before staining. Feulgen's reaction has proved of the greatest worth in the interpretation of the nuclear division of this flagellate. The method described by Robertson (1927) was followed, after fixation with Schaudinn's fluid. The films were hydrolysed at 60° C. for 5–6 min., and the reaction was complete after $1\frac{1}{2}$–$2\frac{1}{2}$ hours in the fuchsin solution. Light green was used as a counter stain. Miss Robertson gives references to the literature upon Feulgen's reaction and discusses it. The reaction consists of a splitting, by partial acid hydrolysis, of the thymonucleic acid in the animal nucleus and the formation of aldehyde groups. These, with the fuchsin-sulphurous acid, give the red colour characteristic of Schiff's aldehyde reaction. The reaction is very specific for chromatin containing thymonucleic acid.

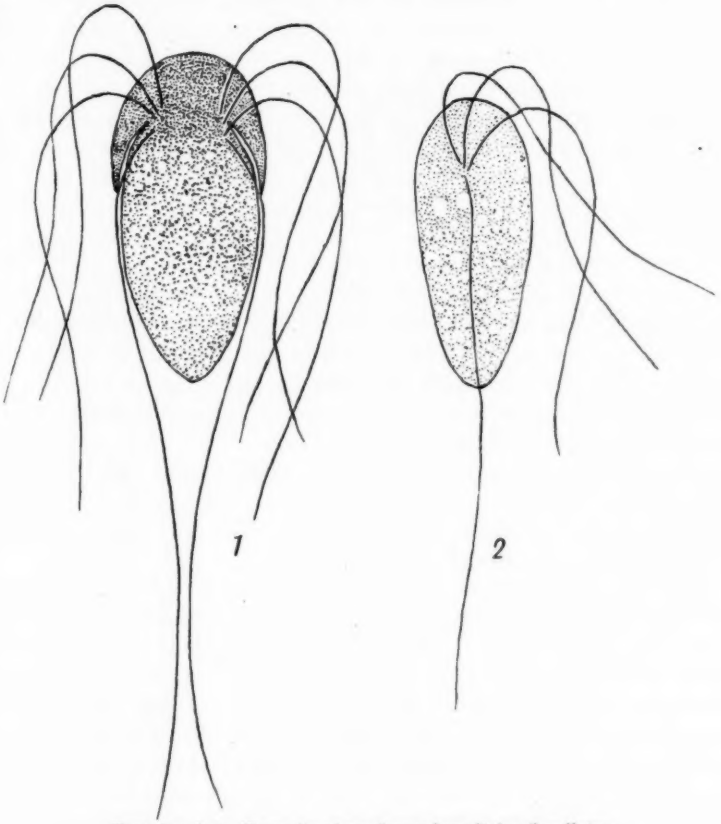
Owing to a slight amount of unavoidable shrinkage and contraction during fixation, fixed and stained preparations, whilst showing the detailed structure of the nuclei and their method of division, and the method of insertion of the flagella, fail to give a correct idea of the shape of the body. The body form and movements of *H. gigas*, therefore, were studied in preparations of the living flagellates in culture solution beneath coverslips which had been well waxed down. In such preparations the flagellate lived for hours. *H. gigas*, like other species of *Hexamita*, moves so rapidly that it is necessary to wait for several hours, until its movements become slower, before it is possible to study it.

III. The structure of *Hexamita gigas*.

Hexamita gigas was found in the hind-gut of the horse-leech and obtained by expressing a little of the content through the anus, or by dissecting the gut. The flagellates were never very numerous, but frequently multiplied very rapidly in the primary cultures. Cysts were never seen in the cultures nor in the hosts. Between 50 and 60 horse-leeches have been examined, but no cysts

have been found, though cysts of *Entamoeba aulastomi* and *Chilomastix aulastomi* frequently were numerous. Cysts have been described from *Hexamitus* (= *Hexamita*) *muris* (Wenyon, 1907) and *Octomitus* (= *Hexamita*) *dujardini* from frogs and toads (Dobell, 1909).

The body of *H. gigas* is elongated, being about 2 or $2\frac{1}{2}$ times as long as broad. The living flagellates vary between $8–14 \times 4–7\mu$, the usual size being $12 \times 6{\cdot}5\mu$. When fixed, the body contracts and becomes more rounded,



Text-figs. 1–2. *Hexamita gigas*, drawn from living flagellates.

varying between $6{\cdot}5–10 \times 5–7\mu$. The anterior end is rounded whereas the posterior tapers slightly, the body being broadest about a quarter its length from the anterior end. About this region of the flagellate a projection of cytoplasm can be seen on either side having the appearance of a pair of flaps closely applied to the body. No such structures have been described in other species of *Hexamita*. They are not seen in fixed and stained flagellates, but are striking in the living specimens (Text-fig. 1). The body is very pliable and

capable of change of form, and is able to squeeze itself through very narrow channels.

There is no mouth in *H. gigas* which, like *Retortamonas orthopterorum* (Bishop, 1932 *a*), ingests bacteria and starch grains at the posterior end of the body.

H. gigas has eight flagella, each arising from a minute basal granule, in two groups of four, on either side the body close to the anterior end (Text-fig. 1; Pl. fig. 2). The basal granules are not stained by Feulgen's reaction, as already found for other protozoa, *e.g. Trypanosoma raiae* and *Bodo caudatus* (Robertson, 1927), *Trichomonas* sp. from the frog (Reichenow, 1928) and *T. batrachorum* (my own unpublished results).

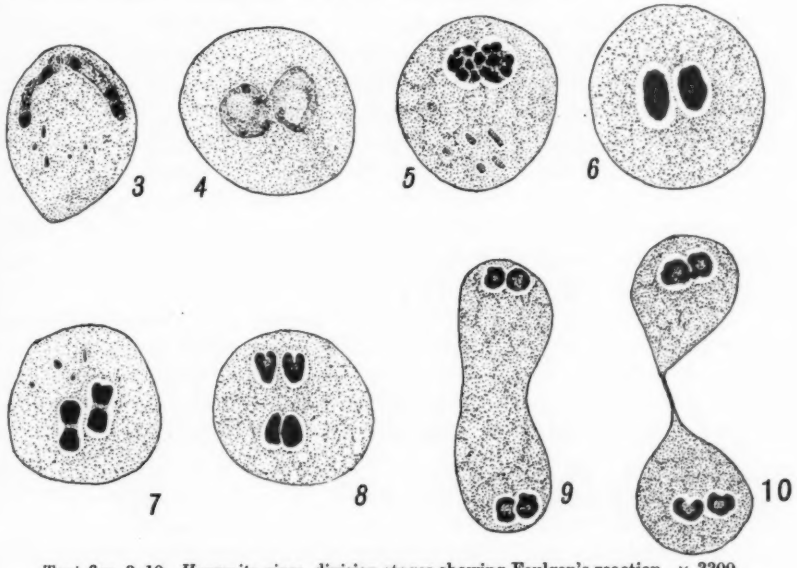
H. gigas swims by means of the three anteriorly placed flagella of each group. The two remaining flagella trail behind and are seldom seen to move. It is characteristic, as observed by earlier workers on the genus, that these two flagella are used frequently for anchoring the flagellates to some substance, whilst the anterior ones continue to beat. The flagella are often twice the length of the body. In fixed and stained preparations the course of the posterior pair of flagella, from their origin in their respective basal granules, can be traced along the body until they project beyond its posterior end. In the living flagellate their position can be seen (Text-fig. 2) as it turns in swimming. Usually these flagella are closely applied to the body wall, but that they are not attached to it was shown by rare observations on several individuals of sudden upward and outward strokes.

In some stained preparations a pair of delicate, faintly staining axostyles was seen lying below the posterior flagella; but in others they could not be found. They never stained with Feulgen's reaction, and were never found projecting beyond the edge of the body.

The arrangement of the flagella, as described above for *H. gigas*, differs from that described in other species. Thus, in *H. muris*, Wenyon (1907) describes the tail flagella as arising from the sides, or close together at the posterior end of the body; in *Octomitus* (= *Hexamita*) *dujardini*, Dobell (1909) describes the caudal flagella as arising from a pair of chromatin granules at the posterior extremities of the axostyles; whilst in *H. ovatus*, *H. intestinalis* and *H. batrachorum*, Swezy (1915) also describes them as arising from the posterior ends of the axostyles.

The arrangement of the six anterior flagella varies in different descriptions. Dobell (1909) describes four basal granules, in close apposition, or connected with each other by a delicate filament of chromatin, lying at the anterior end of the body, two flagella arising from each anterior granule and one from each posterior. Swezy (1915) describes a blepharoplast complex consisting of a pair of granules at the anterior end, from which the six anterior flagella arise, three from each granule. In *Hexamita intestinalis* (= *Octomitus dujardini* Dobell 1909), Wenyon (1926) describes the anterior flagella as each arising from a separate granule, whilst the two axostyles arise from a similar pair.

In the living *H. gigas* (Text-fig. 1) the nuclei cannot be seen, but the anterior end of the body has a much denser appearance than the posterior, which is finely vacuolated. The two nuclei are similar in shape and structure. They are elongated (Pl. fig. 2) and lie in the anterior end of the body. If differentiation is carried far enough each nucleus is seen to be composed of a lightly staining matrix in which lie siderophilic granules. In the majority of individuals two such granules lie one at either end of each nucleus (Pl. fig. 1). The Feulgen reaction (Text-fig. 3) gives a similar picture.



Text-figs. 3–10. *Hexamita gigas*, division stages showing Feulgen's reaction. × 3300.

IV. DIVISION.

Division is very rapid, the first sign being the rounding up of the body. In such rounded forms axostyles were never seen. The flagella of *H. gigas* are extraordinarily fine and difficult to follow, and the attempt to study them during the division process has met with great difficulty. After the body has become rounded the nuclei also become spherical (Pl. fig. 3) and more granular. Nuclei at this stage are difficult to stain with Feulgen's reaction (Text-fig. 4). The granules become very coarse so that each nucleus has a morula-like appearance. The nuclear membrane, still well defined, becomes spindle-shaped, one pair of flagella with their respective basal granules come to lie at either pole (Pl. fig. 4), and the anterior flagella can no longer be distinguished from the posterior. No centrosome is formed from either basal granule of either pair and there appears to be no differentiation of the granules to perform this function. The chromatin in each nucleus becomes an elongated,

very dense mass (Pl. fig. 5), and neither in preparations stained with iron haematoxylin nor in those subjected to Feulgen's reaction (Text-fig. 6) can any structure be detected. As interpreted by Feulgen's reaction each nucleus appears to contain a solid block of chromatin. The nuclear membrane becomes less well defined and gradually disappears. The masses of chromatin divide, though not always simultaneously (Text-fig. 7), across the equator into two blocks. As the two daughter blocks draw apart (Pl. fig. 6) a faintly staining line appears between each pair. This is only seen in preparations stained with haematoxylin. It gradually increases to form an irregularly shaped structure (Pl. fig. 7). As the daughter chromatin blocks of each dividing nucleus become further separated the irregular structures become elongated into broad rods (Pl. fig. 8) which remain separate from the chromatin blocks. The flagellate begins to elongate and an extra basal granule and flagellum appear at the poles of each dividing nucleus (Pl. fig. 9), but how they arise it has been impossible to discover. As the flagellate elongates the rod-like structures between the daughter chromatin blocks elongate further (Pl. fig. 11), become thread-like and stain intensely with iron haematoxylin. In a few individuals these threads then appear to be absorbed (Pl. fig. 10), whereas in the great majority (Pl. figs. 11–13) they intertwine or fuse for the greater part of their length and diverge towards the daughter chromatin blocks. As the body of the flagellate becomes constricted, prior to division, these structures usually persist as a single siderophilic thread differing in appearance from a centrodesmose only in that they are not joined to the basal granules; they differ totally from a centrodesmose in origin. In preparations subjected to the Feulgen reaction (Text-figs. 7–10) such structures are not seen between the well-defined chromatin blocks. They must therefore be of an entirely achromatic nature. In the division of this flagellate, therefore, iron haematoxylin gives a very misleading picture, and if this method alone were used these achromatic structures would probably be interpreted as chromatic.

The fourth basal granule and its flagellum must arise after division of the flagellate, as daughter individuals with complete sets of flagella and basal granules have never been seen whilst still connected with one another.

The axostyles are never seen after the division process begins and they have not been reformed when the daughter flagellates part.

The mode of division in *Hexamita gigas* differs in a number of points from that in many other flagellates. No centrosomes are formed. The basal granules do not divide prior to the division of the nucleus, and no centrodesmose is formed. No chromosomes are present nor is an equatorial plate of irregular chromatin granules ever seen. The chromatin of each nucleus, as interpreted from Feulgen's reaction, appears, in the early stage of division, as a solid block which later divides into two similar but smaller blocks. The nuclear membrane is absorbed. An achromatic element appears between the daughter blocks in each nucleus.

Observations upon the division of species of *Hexamita* by previous workers

are on the whole very incomplete. Wenyon (1907) gives no details of nuclear division in *H. muris*, and I suspect that the stages which he interprets as dividing forms (Taf. 11, figs. 27 and 28) are "double" individuals such as occur in many flagellates but which have not been seen in cultures of *H. gigas*. Dobell (1909) confesses himself uncertain of the method of division of the *Hexamita* of frogs and toads, but the stage which he figures (pl. 3, figs. 40 and 41) agrees with the similar stage (Pl. fig. 9) in *H. gigas*, except that he interprets the achromatic structures between the chromatin blocks as the dividing axostyles. Swezy's (1915) account of the division of *H. ovatus*, *H. batrachorum*, and *H. intestinalis* differ markedly from the description of *H. gigas* given above in that she describes division of the blepharoplasts and formation of a centrodesmose, a splitting of the axostyles, the formation of a mitotic spindle and of two chromosomes in each daughter nucleus. In the division of *H. intestinalis*, Alexeieff (1908) describes the disappearance of the nuclear membrane and the formation of coarse granules in the two nuclei. He also describes the division of the chromatin of each nucleus into two blocks (fig. 4), but he believed each block to consist of 6–8 chromosomes. In *H. gigas*, as stated above, no chromosomes can be seen at any stage of division. He also saw a fairly thick thread, slightly curved, formed of achromatic substance, between the two pairs of separating daughter nuclei.

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EXPLANATION OF PLATE XVIII.

Hexamita gigas.

The figures were drawn with an Abbé camera lucida under a 1/12 in. A. fluorite oil-immersion lens (Leitz) at a table magnification of approximately 3300 diameters. All figures represent preparations stained with iron haematoxylin.

Fig. 1. Flagellate with flagella cut short in the drawing. The stain has been greatly extracted to show the granular structure of the two nuclei.
Fig. 2. Flagella natural length. Nuclei overstained. Basal granules and axostyles shown.
Fig. 3. Rounded up prior to division; granular nuclei.
Fig. 4. Morula-like nuclei; flagella and basal granules arranged at either pole.
Fig. 5. Chromatin of nuclei contracted into solid masses. One chromatin mass has divided. Nuclear membrane absorbed.
Fig. 6. Chromatin masses of both nuclei have divided; the achromatic structure is appearing between the daughter chromatin blocks of each nucleus.
Fig. 7. More advanced stage.
Fig. 8. Achromatic elements becoming rod-like.
Fig. 9. A new flagellum and basal granule has appeared at the poles of each nucleus.
Fig. 10. Achromatic element has been absorbed.
Fig. 11. Achromatic element of each nucleus have become intertwined.
Fig. 12. Body of flagellate becoming constricted before division.
Fig. 13. More advanced stage.

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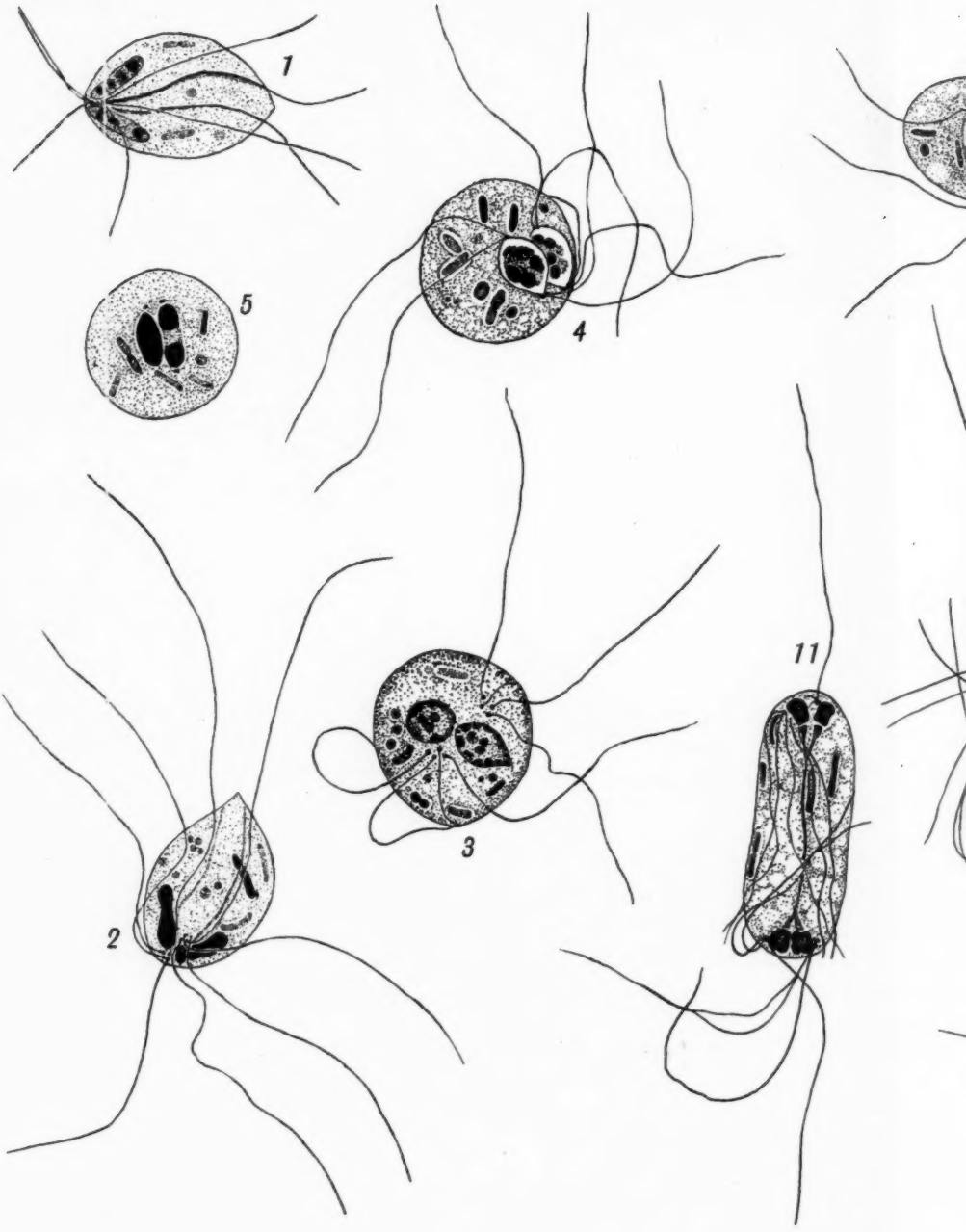
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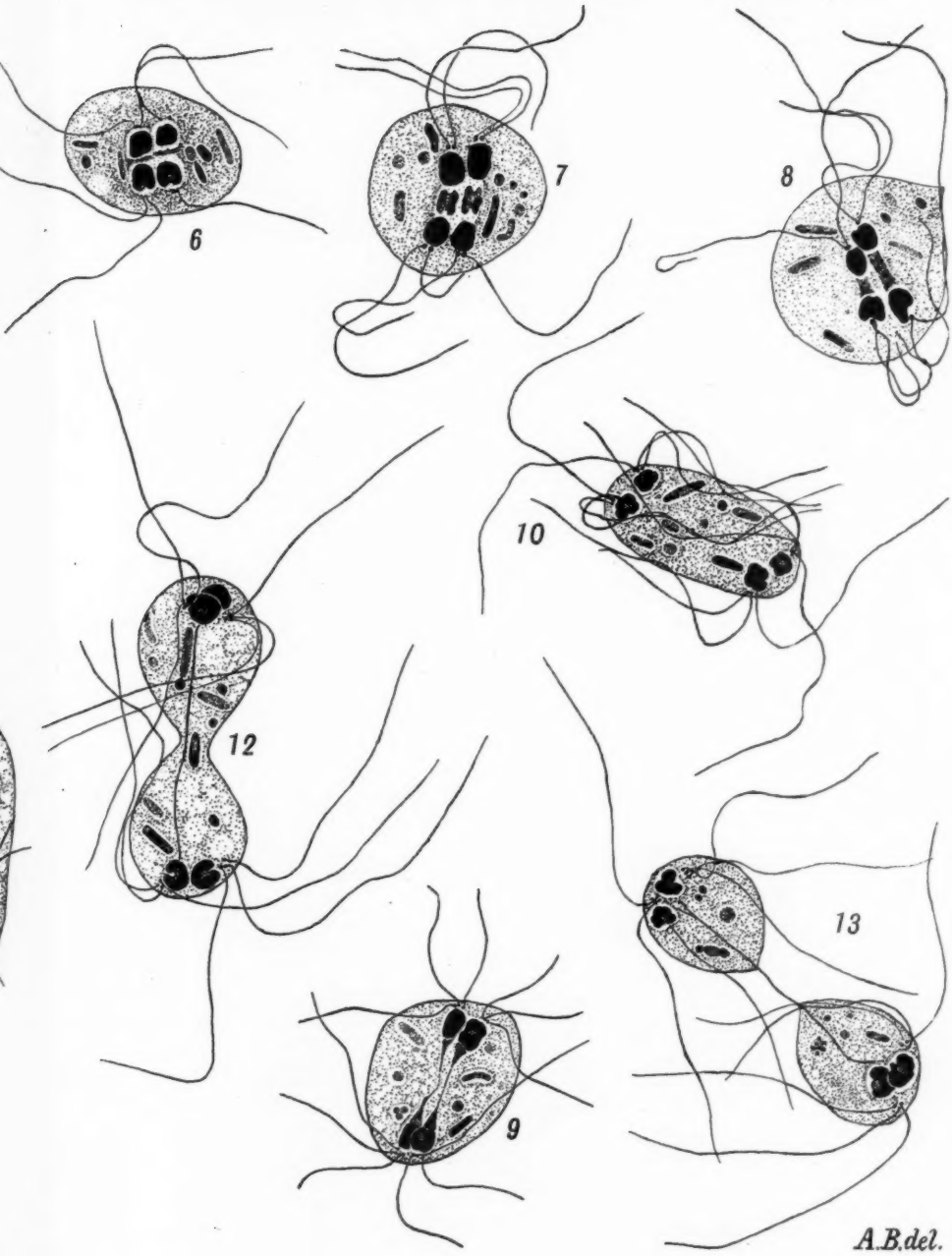
PLATE XVIII



THE DOMESTIC FOWL OF UGANDA AS A HOST FOR TRYPANOSOMES OF THE *BRUCEI* GROUP.

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INTRODUCTION.

For many years it has been known that the domestic fowl, together with some other species of birds, is susceptible to experimental infection by trypanosomes of the *brucei* group. The successful experiments were performed in European laboratories with strains whose virulence had been greatly exalted as the result of long maintenance in small laboratory animals. Infection was induced by inoculation of infected blood into the comb, the peritoneum or a vein. A natural infection of birds with these trypanosomes has, as far as I know, never yet been reported.

The Institute possesses no library, and I am indebted to the Director and Secretary of the Tropical Diseases Bureau for the following brief summary of the work published on this subject other than that done in Uganda.

In 1900 Ziemann unsuccessfully attempted to infect a duck, a fowl and a pigeon with the blood of a dog naturally infected with the Togoland virus (Ziemann, 1902).

In 1902 Schilling infected two geese out of three by inoculation with the pericardial exudate of a dog infected with the Togoland virus (25th dog passage). A hen, duck and pigeon inoculated at the same time remained free

from infection (Schilling, 1904). Up to this time, according to Laveran and Mesnil, all authors with the exception of Vosges were agreed that birds were refractory to the pathogenic mammalian trypanosomes. In October, 1904, Mesnil and Martin repeated Schilling's experiment with fowls and geese, using as virus (1) *T. brucei* (Zululand strain), (2) *T. evansi*, and (3) *T. equinum*. One goose inoculated with *T. brucei* became mildly infected, all the other experiments yielded negative results (Mesnil and Martin, 1906).

In 1906–7 Goebel inoculated blood from guinea-pigs infected with *T. brucei* (Zululand strain) into the caruncles of 23 fowls. Subsequent sub-inoculation into guinea-pigs after from 2 to 55 days showed that the blood of 20 of these fowls remained infective for guinea-pigs for 3–81 days (Goebel, 1906 and 1908).

Durham published in 1908 some observations made by him ten years earlier on the relation of birds to nagana. He had then unsuccessfully attempted to infect pigeons with nagana, but had succeeded in infecting a kestrel by inoculating into its breast citrated rat's blood swarming with nagana trypanosomes (Durham, 1908).

In 1912 Mesnil and Blanchard injected 1 c.c. of the diluted blood of mice infected with *T. gambiense* and *T. rhodesiense* into the wattles of eight fowls, four with each species of trypanosome. All the *gambiense* and three of the *rhodesiense* injected fowls became infected, as shown by sub-inoculation into rats. Trypanosomes were never seen in the daily blood examinations of the fowls, though a few *T. gambiense* were seen on the layer of leucocytes obtained by centrifugation of the blood of one of them at the time of death. Four of the fowls had ocular symptoms (Mesnil, 1912; Mesnil and Blanchard, 1912).

Sollazzo in 1929 investigated the influence of starvation and lack of vitamins on the resistance of pigeons to trypanosome infection. He found that pigeons were normally completely refractory to *T. brucei* infection, but when subjected to complete starvation or fed on white corn from which the antineuritic vitamin had been removed, five out of six pigeons showed a decreased resistance to the trypanosomes—obvious multiplication of the parasite occurring in the peripheral blood of two, and in five of the six inoculation proving positive (Sollazzo, 1929).

Coming now to the Uganda work. In 1909 Sir David Bruce and his coworkers at Mpumu in Uganda took up the study of the domestic fowl as a possible reservoir for *T. gambiense* (Bruce *et al.*, 1911). In Bruce's experiments an attempt was made for the first time to imitate natural conditions by the use of tsetse and freshly isolated strains of trypanosomes. The trypanosomes used were either *T. gambiense* or the lake-shore trypanosomes which at the time were held to be that species.

Thirteen birds were fed upon, each by a separate box of flies. All the boxes save one were proved to be infective to clean monkeys either before and after or immediately after feeding on the fowl. Each box of flies thus contained at least one fly with infected salivary glands. Each bird was thoroughly examined for trypanosomes microscopically, centrifuged blood

being carefully searched and then inoculated into a clean monkey. No trypanosomes were found and no fowl became infected. In a further experiment 400 clean flies were fed on birds that had been bitten by flies known to be infective with *T. gambiense*. The flies were then fed for a sufficient period on a clean monkey, which never became infected. 283 of the flies were dissected and none contained any flagellates.

An indirect result of this investigation was the employment of fowls to nourish the flies used in transmission experiments, during the 2 or 3 weeks' interval that elapses between the infecting feeds and the final test on a clean animal. The flies fed well on the birds, which were readily obtainable in large numbers. At Mpumu each box of flies had its own numbered fowl upon which it was fed during the greater part of the experiment.

A similar investigation was carried out in 1911, at Mpumu, by Fraser and Duke (1912). Some of the trypanosomes used were derived from lake-shore flies, and identical as far as is known with the Damba trypanosome of recent years. The remainder were *T. gambiense* derived from man.

Ten fowls were taken and ten boxes of infected laboratory-bred flies. One box was allotted to each fowl and was fed upon it on alternate days for 4 weeks or more. Immediately after the last feed on the fowl each box was fed on a different clean monkey and all the monkeys became infected. Each bird was thus bitten on several occasions by at least one fly with infected salivary glands and probably, in many cases, by more than one. After a suitable time had elapsed for the appearance of trypanosomes in the fowls' blood, 129 clean flies were fed in turn on each of the ten birds. In due course these flies were all dissected and none found to contain flagellates. Finally, the blood of all the ten birds was inoculated into a series of clean monkeys, none of which became infected.

In 1912 I carried out experiments on similar lines to ascertain if ducks are capable of acting as a reservoir of *T. gambiense*. Infective *Glossina palpalis* were fed on two tame Muscovy ducks which were afterwards tested with clean flies and by blood inoculation into a susceptible animal. The ducks were not found to be infected and were incapable of infecting clean flies (Duke, 1912 *b*).

These experiments supply reasonable proof that, in the conditions of experiment obtaining in Uganda, the fowl cannot serve as a host either of *T. gambiense* or the trypanosomes carried by the lake-shore tsetse.

No strains of *T. brucei* from game tsetse areas or of *T. rhodesiense* were studied at Mpumu. Work at that laboratory closed down in 1912. On the resumption of trypanosome investigations at Entebbe in 1919, the fowl was again used as a food supply for the flies. Until the arrival of the League of Nations Sleeping Sickness Commission at Entebbe, trypanosomiasis research was subordinated to the calls of the ordinary routine work of the Medical Laboratory Services. When the Commission began its work early in 1926, I was liberated for whole-time research, and transmission experiments multiplied apace. The procedure was altered. Instead of a numbered fowl for each

box of flies, some 20–30 birds were kept in a "pool" and used indiscriminately for nourishing the flies during the earlier weeks of their probation. Each fowl was fed on by one box of flies each day. In this way opportunity arose for fowls to be bitten by flies whose glands were infected before the 25th day of their cycle with *T. rhodesiense* or *T. brucei*, the same birds being subsequently bitten by the flies of other boxes devoted to other strains. In the complete absence of any known cause for suspicion the control experiments performed at Mpumu were held to be sufficient warrant for this procedure.

Such was the routine when early in 1931 Dr Corson wrote to me from Tanganyika reporting the successful infection of fowls with strains of *T. rhodesiense* recently isolated from man. He inoculated five fowls in the muscles of the leg with the blood of white rats infected with three strains of *T. rhodesiense*. Two became infected. Trypanosomes were not seen in their blood, but were transmitted by the syringe to white rats in which they multiplied rapidly and showed posterior-nuclear forms. The fowls remained infected for at least 6 weeks and showed no impairment of health (Corson, 1931).

In the light of this announcement it became necessary to carry out control experiments with fowls as carriers of freshly isolated strains of *T. rhodesiense* and *T. brucei* from game tsetse regions, on similar lines to those done at Mpumu with *T. gambiense* and the lake-shore trypanosomes. Two of the principal lines of investigation of recent years at Entebbe have been the rôle of ruminants as a reservoir of the human trypanosomes and the changes in transmissibility exhibited by a trypanosome during a prolonged sojourn in one and the same individual host. To study these points batches of some 400–500 flies, each batch called a "test," are applied at intervals of a month or two to the infected animal. After a suitable time has elapsed the flies are dissected, any flagellates found in them being ascribed to the original infecting animal. It is evident that a casual infection picked up from the fowls of the "pool" would vitiate work of this nature. If trypanosomes are picked up secondarily from the fowls used to nourish the flies the fact will be manifest in the total percentage of positive flies, calculated from the number of gut infections found on dissection, rather than in the number of gland infections. For in a great number of instances the final dissection of the flies takes place before sufficient time has elapsed for a hypothetical infection picked up from the fowl to reach the gland stage. Unless of common occurrence such an error would not be of much importance, merely giving rise here and there to a slightly exaggerated estimate of the capability of a strain of trypanosome to utilise the tsetse. If, however, a complete developmental cycle culminating in infection of the glands can be carried out, within the time allotted to the original transmission experiment, by trypanosomes derived from the fowls the situation will be more serious. The whole question, therefore, deserves very careful investigation.

OBJECTS OF THIS PAPER.

(*To obtain answers to three questions.*)

It is required to determine whether *in the experimental conditions obtaining at Entebbe* the Uganda fowl:

1. Can become infected with *T. rhodesiense* or *T. brucei* by being bitten by tsetse cyclically infected with these trypanosomes.

2. If so infected, the fowl can in its turn infect clean *Glossina palpalis*.

3. If it can infect tsetse, what effect, if any, will this fact have upon the results of previous work at Entebbe?

The first two questions must be answered by direct experiment. They are dealt with in Part I. Part II is concerned with the answer to the third question. In addition to the facts revealed by actual experiment, the solution of this question calls for careful examination of the indirect evidence afforded by the Entebbe Laboratory records of recent years. Section 1 of Part II deals with this indirect evidence; Section 2 with some additional experimental evidence, taken from a totally different investigation, that bears on the present discussion. To the writer the importance of Part II tends to overshadow the other objectives because of its value as a control of the work done at Entebbe during the last 5 or 6 years.

The progress of the investigation was interrupted for some nine months by my absence on leave. It has consumed considerable time and has sadly interfered with other pressing undertakings. But its importance is twofold. Some new light is thrown on the rôle of birds as a factor in the spread of human trypanosomiasis; and secondly, the indirect evidence, the effect of which is very reassuring, would otherwise be inaccessible to the general reader.

PART I. EXPERIMENTAL EVIDENCE BEARING ON QUESTIONS I AND II.

SECTION 1. PRELIMINARY EXPERIMENTS.

On the arrival of Dr Corson's letter in February, 1931, five of the oldest of the birds then in the "pool" were killed and their blood inoculated on 23. ii. 31 into a clean monkey. Fourteen days later the monkey showed *T. rhodesiense* in its blood. This is, I believe, the first occasion on which a fowl has been infected with a pathogenic mammalian trypanosome by the agency of cyclically infected tsetse. On 11. iii. 31, three more birds from the "pool" were killed and their blood inoculated into a clean monkey. The animal did not become infected. On 17. iii. 31, the blood of four more fowls from the "pool" was inoculated into a clean monkey, whose blood showed *T. rhodesiense* for the first time on 27. iii. 31. On 19. iii. 31 five more of the fowls were killed and their blood inoculated into a monkey. This animal did not become infected.

Selected and numbered clean fowls were now fed upon by boxes of flies, each box being proved by subsequent dissection of the flies to contain one

or more with infected glands. In this series of experiments fowls 1*a*, 1*b*, 1*c*, 1*d*, were exposed to flies carrying *T. gambiense*, and fowls 1, 2 and 3 to flies carrying *T. rhodesiense*. After allowing for an incubation period of at least 14 days, clean flies were fed on these fowls, and a week or so later the blood of all the birds was inoculated into clean monkeys. None of the flies and none of the monkeys became infected. In these preliminary experiments time pressed and the experiments had to be terminated rather earlier than was desirable. In all, 16 gland-infected flies carrying *T. gambiense* and 23 carrying *T. rhodesiense* were found among the flies that fed on the fowls in the two groups. Each bird was fed upon by at least three boxes of infected flies. The distribution of infective flies in the various experiments and the age—that is the date in the cycle—of the flies at their last feed on a fowl were as follows:

T. gambiense flies.

Exp. No.	Last feed on a clean fowl	Gland-infected flies	Exp. No.	Last feed on a clean fowl	Gland-infected flies
226	22nd day	2	231	21st day	3
225	31st „	1	243	24th „	3
230	22nd „	2	256	23rd „	5

T. rhodesiense flies.

234	20th day	4	247	23rd day	1
235	22nd „	1	248	23rd „	1
236	25th „	3	255	21st „	1
241	25th „	1	258	21st „	1
242	18th „	1	259	21st „	2
244	24th „	2	261	19th „	1
245	24th „	2	264	23rd „	2

The final dissection of flies in each box took place 48 hours after the last feed on the clean fowl, the number of gland-infected flies in each box being determined at this final dissection. The weakness of this series of experiments lies in the age of the flies when applied to the birds. It is possible that some of the gland infections revealed on final dissection had not developed when the box was actually applied to the bird. On the other hand the fly in Exp. 242 of the foregoing list infected a guinea-pig upon which it fed on the 20th day of the cycle. The details of the application of clean flies to these birds is shown in Table II, Section 1.

Section 2. Final experiments.

As in the preliminary experiments, the fowls were exposed to infection by feeding upon them flies whose glands were infected with the particular polymorphic trypanosome which it was desired to test. The majority of the experiments were performed with *T. rhodesiense* and *T. brucei*; a few with the Damba trypanosome. No attempt was made to infect birds by means of direct inoculation of infected blood, as this had already been done by others. A suitable time after its exposure to the infected flies each fowl was examined to see if it showed any sign of infection. For this purpose reliance was placed

mainly on the inoculation of the bird's blood, mixed with citrate, into a clean monkey. The infectivity of the flies used to infect the birds was demonstrated by dissection, and also in a number of cases by the infection of clean animals by the bite of the same flies. In estimating the degree of exposure of the birds to infection relevant points are the number of flies with infected glands and the age of the flies at the time they were feeding on the fowls. This information is set out in Tables I and II. It is also important, as the experiments themselves demonstrated, to note the time elapsing between the actual exposure of the birds to infection and the application of the tests with clean flies. This is also shown in Table II.

A number of birds, after they had been exposed to infection, were fed upon by clean tsetse to see whether the flies could pick up the trypanosomes from them. These clean flies were applied irrespective of whether actual

Table I.

Fowl No.	Species of trypanosome	Exposure to gland-infected flies: Number	Exposure to gland-infected flies: Age in days*	Date of last feed of infective flies on fowl: Year 1932	Sub-inoculation of fowl's blood: Date Year 1932	Animal	Result	Remarks
5	*T. brucei*	1	26	13. i	16. ii	Rabbit	–	Cf. Table II
6*a*	—	1	25	29. i	23. iii	,,	–	Cf. Table II
11	—	1	28	15. i	(1) 16. iii	,,	.	— —
					(2) 20. v	Monkey	–	— —
12	—	1	30	11. iii	20. v	,,	–	On 28th day this fly infected a monkey. Bird killed and 7½ c.c. blood inoc. Cf. Table II
13	—	1	30	17. i	(1) 3. ii	,,	+	(1) 1½ c.c. blood from vein
					(2) 18. v	,,	–	(2) Bird killed; 10 c.c. blood inoc. Cf. Table II
17	—	1	29	20. i	9. ii	,,	–	— —
21	—	2	26	8. vi	17. vi	,,	–	Bird killed; 10 c.c. blood inoc.
22	—	1	30	18. iv	20. iii	,,	–	— —
26	—	1	26	4. vi	17. vi	,,	–	Bird killed; 10 c.c. blood inoc.
29	—	2	36	21. vi	16. vii	,,	–	Bird killed; 10 c.c. blood inoc.
31	—	2	38	21. vi	22. ix	,,	–	Bird killed; 10 c.c. blood inoc.
33	—	3	30	13. vi	5. vii	,,	–	— —
16	*Damba T.*	1	30	13. iv	25. iv	,,	–	Cf. Table II
19	,,	1	36	10. iv	23. iv	,,	–	Clean monkeys were infected by this fly on 28th and 38th days. Cf. Table II
5*b*	*T. rhodes.*	12	30	26. v	(1) 18. vi	,,	–	— —
					(2) 18. vi	,,	–	(2) Killed; 10 c.c. blood inoc. Cf. Table II
6*b*	,,	7	33	4. vi	(1) 17. vi	,,	–	— —
					(2) 22. ix	,,	–	(2) Killed; 10 c.c. blood inoc. Cf. Table II
7	,,	1	38	18. vi	16. vii	,,	–	— —
20	,,	1	36	17. vi	16. vii	,,	–	— —
25	,,	1	28	4. vi	18. vi	,,	–	— —
27	,,	5	32	14. vi	(1) 5. vii	,,	+	(1) 1½ c.c. blood from vein
					(2) 18. viii	,,	–	(2) Bird killed; 8 c.c. blood inoc. Cf. Table II
28	,,	4	45	10. vi	(1) 8. vi	,,	–	(1) 1½ c.c. blood from vein
					(2) 16. ix	,,	–	(2) Killed; 6 c.c. blood inoc. Cf. Table II
34	,,	2	28	13. vi	(1) 16. vii	,,	–	(1) 2 c.c. blood from vein
					(2) 22. ix	,,	–	(2) Killed; 4 c.c. blood inoc.
38	,,	1	34	21. vi	22. ix	,,	–	Killed; 10 c.c. blood inoc.
39	,,	1 *m*†	34	10. x	12. xi	,,	–	Killed; 10 c.c. blood inoc. Cf. Table II
42	,,	1	30	27. vi	2. viii	Rabbit	–	Killed; 10 c.c. blood inoc. Cf. Table II
57	,,	2 *m*	44	29. x	—	—	.	Cf. Table II
68	,,	1 *m*	34	11. x	—	—	.	This fly infected a clean mammal before and after feeding on fowl 68. Cf. Table II
71	,,	3 *m*	25	3. x	—	—	.	Cf. Table II
72	,,	1 *m*	42	30. x	—	—	.	Cf. Table II
84	,,	1 *m*	32	15. x	—	—	.	Cf. Table II
88	,,	1 *m*	28	12. x	5. xi	Monkey	+	Killed; 6 c.c. blood inoc. Fowl sick. Cf. Table II

* "Age in days" denoting time elapsed from first infecting feed of fly.
† *m* = *Glossina morsitans* employed, the others were *G. palpalis*.

evidence was forthcoming that the birds were indeed infected. As many fowls as possible of those proved to have been thoroughly exposed to infected flies were examined in this way.

Table I shows the sub-inoculations performed with the blood of fowls that had been exposed to cyclical infection with *T. rhodesiense*, *T. brucei* or the Damba trypanosome.

The age of the flies in column 4 is calculated from their first infecting feed. It may be accepted that gland infections are usually well established by the

Table II.

Fowl No.	Species of trypanosome	Exposure to clean flies: Number	Exposure to clean flies: Period of application to fowl	Date of last feed of infective flies on fowl	Result
Prelim. Expts. (year 1931):					
I *a*	*T. gamb.*	284	10. iii–3. iv	14 days before	No infected flies found
I *b*	,,	254	11–31. iii	—	,,
I *c*	,,	219	3. iii–1. iv	—	,,
I *d*	,,	261	13. iii–1. iv	—	,,
I	*T. rhod.*	216	16. iii–1. iv	14 days before	,,
II	,,	210	16–26. iii	,,	,,
III	,,	210	21–30. iii	,,	,,
Final Expts. (year 1932):					
5	*T. brucei*	52	11–13. vi	13. i	No infected flies found
6 *a*	,,	308	3–16. iii	29. i	,,
12	,,	381	16–29. iii	11. iii	,,
13	,,	311	1–17. iii	17. i	Fowl + on sub-inoculation
16	*Damba T.*	106	30. v–6. vi	13. iv	No infected flies found
19	,,	139	6–13. vi	10. iv	,,
5 *b*	*T. rhod.*	416	6. vii–14. viii	26. v	,,
6 *b*	,,	302	9. vii–28. viii	4. vi	,,
27	,,	299	10. vii–10. viii	14. vi	Fowl + on sub-inoculation
28	,,	302	8. viii–29. viii	10. vi	No infected flies found
39	,,	311	31. x–11. xi	10. x	3 + flies: 2 light and one heavy infection
57	,,	293	1–12. xi	29. x	No infected flies found
68	,,	189	1–13. xi	11. x	,,
71	,,	322	18–2. xi	3. x	,,
72	,,	182	3–10. xi	30. x	1 + fly found: a light infection
84	,,	181	3–14. xi	15. x	No infected flies found
88	,,	245	28. x–5. xi	12. x	1 + fly: a heavy infection: fowl + on sub-inoculation

Total preliminary experiments = 1654. Total final experiments = 4339.

25th day and probably many flies carrying *T. rhodesiense* are actually infective a few days before this date. The letter *m* in column 3 means that the fly was *G. morsitans*; all the others were *G. palpalis*. The date in column 5 is that of the last feed on the fowl. In every case the infective fly or flies had fed upon the fowl on at least six occasions previous to this date, *i.e.* on alternate days from the 12th day of each experimental cycle. In the case of the older flies the period on the numbered fowl was of course considerably longer.

Table II shows the results obtained by feeding clean laboratory-bred *G. palpalis* on certain of the fowls of Table I.

The period elapsing between the last exposure of each bird to gland-infected flies and the application of the clean flies is seen at a glance.

The flies in most of these experiments were killed between the 15th and 20th days after feeding on the numbered fowls. No attempt was made to transmit trypanosomes cyclically from bird to monkey. What was wanted was an answer to the question: Can the fowl infect clean flies? It seems reasonable, however, to assume that sooner or later a gland infection might have been obtained had a large enough number of flies been fed on birds such as Fowls 39 and 88. Unless the contrary is stated in the last column, no evidence of infection of the fowl was obtained.

Table II shows that Fowl 13 failed to infect any of the 311 flies fed upon it between March 1st and 17th. On February 3rd the blood of this fowl infected Monkey 982 whose blood was then inoculated into Monkey 916. The results of a test of clean flies applied to Monkey 916 was as follows:

Date (March)	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
10–31	359	10	2·7	3	4	2·0

The strain of *T. brucei* infecting Fowl 13 had been previously shown to be considerably less transmissible than either of the two strains of *T. rhodesiense* used to infect Fowls 39 and 88. Of the clean flies fed on Fowl 39 0·32 per cent. and on Fowl 88 0·40 per cent., showed flagellates in the gut. It is evident, therefore, that in comparison with the monkey the fowl is an exceedingly poor infector of tsetse.

Comments on the tables.

(1) The majority of the fowls in these experiments, although bitten on several occasions by flies carrying heavy gland infections of *T. rhodesiense* or *T. brucei* never showed any sign of infection; their blood was neither infective to susceptible monkeys nor to clean laboratory-bred *G. palpalis*.

(2) Seventeen fowls were thoroughly exposed to infection each with one of five different strains of *T. rhodesiense* recently isolated from man, and in only four of these birds was evidence of infection obtained. Of these four only Fowl 88 infected both a clean fly (one out of 245 fed upon it) and a clean monkey (cf. tables); Fowl 39 infected three flies out of 311 fed on it, two of these infections being abortive and limited to one or two trypanosomes in the whole gut contents in 10-day-old flies; Fowl 72 infected one fly only out of 182 fed on it, the infection being limited to the gut and exceedingly scanty; and Fowl 27 was found to be infected by blood inoculation only, and failed to infect any of the 299 clean flies fed upon it at about the same time that its blood was proved positive. The heavy exposure of Fowls 5 *b* and 6 *b* in Table I should be noted.

Of 12 fowls that were exposed to flies infective with the Kazinga strain of *T. brucei* one only was proved to be infected—by blood inoculation. 311 clean flies were fed on this bird and none became infected.

Of two fowls exposed to infection with the Damba trypanosomes neither became infected.

Exclusive of the preliminary experiments, altogether 4339 flies were fed upon fowls that had been well exposed to infection with *T. rhodesiense* or *T. brucei*. All these flies were dissected and five were found to be infected. Two of these five flies showed normal heavy gut infection; in the other three the infection was extremely slight and, in all probability, abortive (cf. Part II, Section 1 (*c*)). This infection rate is in strong contrast to the normal behaviour of these two trypanosomes in susceptible mammals, when exposed to fly.

(3) Blood from Fowl 13 (*T. brucei*) infected a monkey. Clean flies, fed on this fowl-to-monkey strain, readily transmitted the trypanosome cyclically to another clean monkey. The transmissibility of this strain appeared to be unaffected by its sojourn in the fowl.

(4) The three abortive infections described suggest that while in the fowl's blood the transmissibility of the trypanosome is in some way inhibited. Whether this is due to a physiological condition of the trypanosome or merely to its extreme rarity in the bird's blood, it is impossible to say. These three infections were so very slight that in all probability the flagellates would have died out had the flies lived on longer.

(5) The two normal gut infections were found in flies that had fed on a fowl shortly after its exposure. It would thus appear that the fowl can only infect *G. palpalis* during the early stages of its infection and even then to a very feeble extent.

(6) The behaviour of Fowl 27, whose blood 44 days after having infected a monkey failed, on a second sub-inoculation, to produce infection, suggests that an infection of the fowl caused by the bite of a tsetse may soon die out.

(7) Only one of all the exposed fowls showed any signs of illness, *i.e.* Fowl 88, and this bird infected a fly at about the same time that injection of 10 c.c. of its blood infected a clean monkey. It is, however, impossible to decide whether the indisposition of this fowl was attributable wholly or even in part to the trypanosome infection.

(8) That trypanosomes are indeed exceedingly rare in an infected fowl is shown by the failure of the blood of Fowl 39 to infect a susceptible monkey, although the inoculation was done at approximately the same time that the three flies had picked up trypanosomes from this bird's blood.

Section 3. On the significance of light infections of tsetse with trypanosomes of the polymorphic group.

In the foregoing comments (p. 179) certain fly infections are described as abortive, and it should be explained why this view, with the implications that ensue from it, is adopted.

In the experiments recorded in Table II three flies were found to contain each an extremely small number of flagellates in the intestine. In two out of the three actually but one or two flagellates were seen in the teased-out

gut contents: in the third fly a score or so were scattered through the preparation. Very slight infections of this type in flies that have taken more than one clean meal after their infecting feed have been noted from time to time at Entebbe.

Lloyd, working in Nigeria, also described light infections that were "likely to die out," in the gut in *G. tachinoides* in his transmission experiments, though whether he includes in this category the exceedingly slight kind of infection we are now considering is not certain (Lloyd, 1930).

Light infections in laboratory-bred tsetse may be due to three causes:

(1) They may represent passive survival, with perhaps a very little multiplication, of the trypanosomes taken in during the infective meal. Robertson (1913) mentions this phenomenon, which is recognisable by the presence of the apparently unaltered red blood corpuscles of the original infector. Infections of this kind will in all probability die out when all the blood of the original meal has been digested. They are thus confined to the early days of the cycle.

(2) They may be due to the tsetse picking up trypanosomes from the "clean" animal upon which the flies are tested at the end of the transmission experiment. In a positive transmission trypanosomes will, of course, be present in the peripheral blood of this animal before they are actually recognised in the routine daily blood examination. This second kind of light infection will thus be met with at the final dissection of the flies at the end of the test.

(3) The third kind of light infection, also revealed late in the course of an experiment, is the one we are considering. It is obviously acquired at the infecting meal, and survives after the digestion of the blood in which the trypanosomes were originally imbibed. Such an infection may persist at all events to the 31st day after the infecting feed.

Lloyd distinguishes between "mature infections" and heavy infections of the gut only, "likely to become mature." In the former the glands are involved, in the latter they are not. He thus appears to regard gut-only infections in flies dissected late in the cycle as potentially "mature" in that the flagellates may, if given time, eventually invade the glands.

My own view on this point is different. I believe that if flagellates do not reach the glands within a certain fairly well-defined period of time after the original infection of the fly they never will do so. The period that elapses before the glands become invaded in a normal cycle in the tsetse varies somewhat in different conditions of experiment. Under the conditions of experiment obtaining at Entebbe it is about 25 days. Occasionally the glands are invaded earlier than this. What the factors are that determine this variation is at present the subject of investigation at Entebbe and no doubt elsewhere. Climate, the species and perhaps the race of tsetse, and the qualities of the trypanosomes may all play a part. I am aware that several investigators, including Kleine in Tanganyika, have found on occasion that experimental flies which fail to infect a susceptible animal, say between the 25th and

30th day of the cycle, may infect during the ensuing 5 or 10 days. But feeding flies on a clean animal is not such an accurate test of gland invasion as dissection of the flies; and in my experience flies carrying very light gland infections do not always infect the animals they bite. Had Kleine's flies been dissected after their failure to infect between the 25th and 30th days it is quite possible that slight gland infections would have been found. Other observers who have reported the same phenomenon have calculated the date upon which the fly becomes infective by deducting a certain number of days for the incubation period in the test animal. This period, in my experience, is subject to variation, which will materially affect this calculation. My attitude in this matter is based on the experience of a number of years at Entebbe, embracing scores of transmissions with many different strains of trypanosomes and involving, at a very conservative estimate, the dissection of 100,000 experimental tsetse. Both by dissection and by testing on clean animals it has been established beyond doubt and by several investigators that a considerable proportion of the infected flies in any series of transmission experiments with either *T. gambiense*, *T. rhodesiense* or *T. brucei* never develop gland infections, however massive may be the infection of the gut. Failure to reach the glands occurs more commonly in some strains of trypanosomes than in others, a statement in support of which a considerable amount of proof is still being accumulated. *T. rhodesiense* is as a general rule better able to infect tsetse than is *T. gambiense*, and indeed there are in nature strains of the latter trypanosome that are actually incapable of being transmitted by *G. palpalis*. All these observations tend to show that there are considerable differences between different strains in their power to use tsetse; and, in their effect upon the survival of a trypanosome in nature, there is no difference between a normal heavy gut-only infection and one of the three meagre ones described on pp. 180–1. In both the trypanosome has failed to reach the glands. In neither case can the fly transmit the trypanosome to another animal.

It is difficult to prove the correctness of this view, but the observations upon which it is based are as follows. First of all it must be understood that these very light infections, occurring in flies dissected more than a week after their last infection feed, are, in my experience, very rare. In the very great majority of positive flies dissected in the course of many transmission experiments with many different kinds of strains the flagellates are present in large numbers in the intestine, whether the strain is transmissible or not. There are degrees of intensity of infection which may be indicated by the symbols "+++" (the normal heavy gut infection) and "++" respectively: but the "++" infection, which is itself unusual but which may be associated with an infection of the glands in the same fly, is something very different in appearance from the light or "+" infections now under discussion.

About the end of 1920 and early in 1921 an antelope strain of the Damba trypanosome was tested in the laboratory with *G. palpalis*. Of 731 flies

dissected, 2·32 per cent. contained flagellates and 0·68 per cent. showed gland infection (Duke, 1923).

The strain was maintained by direct syringe passage from monkey to monkey and was retested with *G. palpalis* in November, 1922. 524 flies were dissected and 2·2 per cent. showed gut infections; none showed gland infections and the flies were non-infective to clean monkeys. Maintenance by syringe passage in monkeys was still continued and finally in December, 1923, another fly test was applied. In this last test 977 flies were dissected and none of them contained any flagellates.

The experience with this antelope strain was repeated with a strain of *T. rhodesiense* obtained from man in the Mwanza epidemic, in August, 1922. When first tested in September, shortly after isolation, 421 flies were fed on a monkey carrying the strain; 31 were subsequently found to be infected and 22 of them had gland infections. Three of these 31 infected flies showed light gut infections designated "+," as distinct from "+++," the symbol for a normal heavy infection. It was noted in the accompanying text that these were interpreted as an indication that the strain had already undergone slight modification in relation to tsetse during its second monkey passage from man. Eleven months later the strain was again tested, after eight direct passages in monkeys. 1482 flies were dissected of which six contained flagellates. The age of these six flies on dissection were respectively 9, 10, 10, 11, 22 and 31 days. "In every case the organisms were exceedingly scarce, amounting to a few individuals in the whole preparation of the teased-out gut." There were no gland infections (Duke, 1924).

Light infections in the sense employed here seem, therefore, to possess a definite significance; and their appearance is an indication that all is not well with the strain in its adaptation to its insect host.

The minute infections observed in the three flies fed on Fowls 39 and 72 might be ascribed to the inbibition of very minute numbers of trypanosomes by the flies. But this is not at all a likely explanation. Hoare (1931) has estimated that a tsetse feeding on a crocodile infected with *T. grayi* will take up from one to six trypanosomes only per meal. *T. gallinarum* is very rare in the blood in fowls and so is *T. melophagium* in the sheep, and yet heavy infections of these trypanosomes occur in the tsetse and the ked respectively. Again, in the antelope of the Mpumu experiments *T. gambiense* was exceedingly rare in the blood, but these light infections were never observed.

It appears to be more probable, as already suggested above, that they are ascribable to some inhibiting influence exercised on the trypanosome by a host biologically unsuited to its requirements. The incompatibility may be due to a quality inherent in the species of host; or it may be a property acquired by the trypanosome as a result of changes in its constitution consequent on exposure to adverse influences.

PART II. INDIRECT EVIDENCE BEARING ON QUESTION 3 (p. 175).

Indirect evidence bearing on the third of our questions is to be found in a study of the experimental records of the last few years. It may be derived from three sources:

1. Experiments devised to determine whether the prolonged sojourn of a trypanosome in one and the same animal diminishes the transmissibility of that strain by tsetse.

2. Experiments with strains known to be very slightly or not at all transmissible, carried out at a time when the intensity of exposure of the pooled fowls to *T. rhodesiense* was at a maximum.

3. Experiments on the ruminant reservoir.

Section 1.

(*a*) *Prolonged infection experiments.*

For some years past at Entebbe data have been accumulated and in part published pointing to the conclusion that, in certain conditions, prolonged sojourn of a trypanosome in one and the same animal leads to a reduction in the transmissibility of the strain by tsetse. The results obtained along these lines have been on the whole remarkably consistent, each successive batch of flies applied to the animal exhibiting a diminished percentage of infected flies. This phenomenon is very well illustrated by the behaviour of Strain XXIX (European) (Duke, 1930). It is evident without any expatiation that the regular appearance of this phenomenon over several years of experimenting with a number of different strains constitutes a very strong piece of evidence in the present enquiry. If infections were being picked up by the flies from the fowls, sooner or later the manifestation of regular progressive diminution of transmissibility must inevitably have been disturbed.

With Strain XXIX 4612 flies were dissected in different tests, and not one of them showed a gland infection. All these flies were nourished for at least 3 weeks on the fowls of the pool, and several of the tests, as is shown in the next paragraph, were carried out during periods of maximum exposure of the birds to flies with *T. rhodesiense* in their glands.

(*b*) *Periods of maximum exposure.*

In the course of the investigations of the last year or two in Uganda there have been three periods when an unusually large number of flies with glands infected with *T. rhodesiense* were being fed daily upon the pooled fowls. Ample experience indicates that, at Entebbe, the glands of laboratory-bred *G. palpalis* destined to become infective with *T. rhodesiense* will contain trypanosomes at the latest by the 25th day after the infecting feed. It is thus possible to estimate roughly the time when the fowls, some or all of which had been fed upon by these infective flies, were most likely to have

trypanosomes in their blood, allowing for an incubation period of 10 days after the infecting bite. In this way a period of 2 or 3 weeks can be chosen in connection with each of these three spells of intensive exposure to *T. rhodesiense*, when the birds were most likely to be carrying trypanosomes in their blood. By examining the records of transmission experiments with strains of known low infectivity to fly, which were being fed daily upon the pooled birds during one or other of these selected periods, the occurrence of contaminative infections should be detected. For example, an untoward increase in the percentage of infected flies in these "control" tests would at once arouse suspicion of contamination from the fowls of the pool.

Associated with the first of the selected periods 20 flies with salivary glands heavily infected with *T. rhodesiense* had fed upon the fowls for some 14 days. During the ensuing two or three weeks five separate tests were in progress, four with Strain XXIX and one with Strain (*b*), both strains known to be non-transmissible by *G. palpalis*. The flies of these tests were fed daily on the fowls of the pool. The figures obtained on dissection of these flies were respectively:

With Strain XXIX, Test	1.	456 flies:	1·3 %	gut infection;	no gland infections
,,	2.	425 ,,	1·4 %	,,	,,
,,	3.	375 ,,	1·6 %	,,	,,
,,	4.	525 ,,	0 %	,,	,,
With Strain (*b*), Test	5.	353 ,,	0·2 %	,,	,,

In these figures there is plainly no suggestion of any contaminating infection of the flies acquired from the birds of the pool. They are characteristic of the two strains and differ widely from the much higher percentages obtained with *T. rhodesiense* transmission experiments running concurrently under identical conditions.

The second selected period of high exposure of the birds to *T. rhodesiense* commenced with the application to the fowls of the pool of 60 gland-infected flies carrying this trypanosome, each fly feeding some six times on one or other of the birds. The boxes containing these 60 flies were nourished on the fowls for an unusually long period, being fed on the birds right up to the final dissection. Here we have four "control" tests: one with a later passage of the Strain XXIX just cited, one with another non-transmissible strain (*c*), and two with yet another strain of known low transmissibility (*d*). The percentage of infected flies found in these tests are as follows:

With Strain XXIX (*a*)	605 flies	0·1 % infected gut:	including no flies with infected glands
(*d*)	581 ,,	0·5 % ,,	including 1 fly with infected glands
(*d*)	582 ,,	1·0 % ,,	including 3 flies with infected glands
(*c*)	634 ,,	0·1 % ,,	no flies with infected glands

Here again there is no reason for suspicion. The figures correspond perfectly with the regular behaviour of these particular strains.

The last of the selected periods comprised 33 flies with glands infected with *T. rhodesiense*. The fowls bitten by these flies were examined by inoculation of their blood into monkeys before my departure on leave in April, 1931. The details have already been given in Part I, Section 1.

(*c*) *Experiments on the ruminant reservoir.*

The early work done at Mpumu on the rôle of antelope as a reservoir of *T. rhodesiense* (Duke, 1912 *b*) is not in any way affected by the present discussion. As has been shown, the research done in recent years at Entebbe on the diminution of transmissibility displayed by trypanosomes under certain conditions of maintenance is also essentially free from criticism. For it is obvious that any casual infection of the flies picked up from the pooled fowls must inevitably have upset the even course of these experiments. The consistency with which the transmissibility gradually diminished in the considerable number of strains tested, and, more significant still, the absence in test after test of any gland infections among the large numbers of flies fed upon non-transmissible strains such as No. XXIX, etc., all constitute very strong evidence against the operation of any fallacy. The case is, however, not so clear in the investigation of domestic ruminants as a reservoir (Duke, 1931). Not that there is anything startling or suspicious in the results obtained. Moreover, if fallacy can be excluded from the other two lines of investigation there is no reason to suppose it enters in here. The Mpumu investigations, carried out under unimpeachable conditions, showed that antelope may remain infective with *T. gambiense* for at least 22 months. In the recent work under discussion six sheep were found to be still capable of infecting the glands of tsetse with *T. gambiense* 12 months or more after the animals were originally infected. The actual results were as follows (Duke, 1931):

Sheep No.				
161	infected the glands of *G. palpalis*		15½ months	After its original infection
218	,,	,,	18 ,,	
173	,,	,,	12 ,,	
216	,,	,,	18 ,,	
316	blood infected clean monkey		32 ,,	
458	,,	,,	15 ,,	

With Sheep 161, 218, 173 and 216 clean flies were used in the test and the transmissibility of each animal's trypanosomes to fly was determined by dissection and the finding of a fly with infected glands. With Sheep 316 and 458, the blood of the sheep was first inoculated into a clean monkey to which clean flies were applied when it became infected. These two latter animals were thus proved to be still carrying a pathogenic trypanosome respectively 2¾ and 1¼ years after their original infection. Moreover, the monkey inoculated from Sheep 316 lived 227 days, and this points unmistakably to infection with *T. gambiense* and not with *T. rhodesiense*, the only possible interloper from the fowls in the pool.

The records of Sheep 161 are still more instructive. The single gland-infected fly in the last positive test applied to this sheep (*i.e.* 15 months after the animal's original infection) infected Monkey 422. This monkey, a large and powerful animal, lived for 646 days; its death was due to accident, and

at the time the animal was still in fairly good condition. The blood of this monkey, examined over a considerable period, gave consistently strong positive adhesion with *T. gambiense* only. Monkey 624, infected from Monkey 422 by fly, lived 144 days (again a *gambiense* reaction) and behaved in the adhesion test always as *T. gambiense*. It must be understood that the details quoted in support of the authenticity of these few experiments are not specially selected to the exclusion of others less favourable. At the time there was no cause for suspicion and no special precautions were deemed necessary to verify the identity of the trypanosome. The fact that all the available evidence bearing on the identity of the strain recovered by the last positive test with Sheep 161 tells so strongly in favour of *T. gambiense* is, I think, ample justification for the conclusions originally drawn from the behaviour of this animal.

As stated in the original publication the realisation that Sheep 161 was still infective to fly was to some extent a surprise, as the penultimate test had been negative and it was thought that the animal was no longer infective. It is of interest, therefore, to note that after this last positive test no less than 3127 clean flies were fed on Sheep 161 during the ensuing 3 years without a single gland infection resulting. And during this period there were always some *T. rhodesiense* fly experiments being fed upon the fowls of the pool.

At the time of the last positive test the animal was evidently near the end of its infective phase. In point of fact superinfection of this sheep with *T. gambiense* by means of *G. palpalis*, carried out immediately after this positive test, produced no demonstrable effect, the 3127 flies being fed on the sheep *after* this superinfection.

There are two other occasions where unexpected results caused surprise, and in the present inquiry any such occasion must be scrutinised. One of these was the behaviour of the wild pig "Horace" (Duke, 1931). This animal infected the glands of a clean fly that fed on it 36 days after the original infection of the pig with *T. gambiense*. Thereafter, it is true, of 1231 clean flies fed on the pig only two developed infections and these were confined to the gut. Several observers, including Fraser and myself, have in the past failed to infect wild pig with *T. gambiense* (Fraser and Duke, 1912). Is it necessary on this account alone to regard this experiment with suspicion? I think not. The wild pig has lately been proved to be susceptible to infection with *T. rhodesiense* by fly-bite, although undoubtedly it is relatively resistant to both the human trypanosomes.

The other instance was the response of the blood of certain individual infected monkeys to more than one species of trypanosome when examined by the red cell adhesion test (Duke and Wallace, 1930). Here, indeed, is a situation that might tempt the critic. The blood of Monkey 612, infected by syringe inoculation from the "reservoir" Sheep 458, normally gave 100 per cent. adhesion with *T. gambiense* trypanosomes. On one occasion out of several trials it gave also 100 per cent. response with the Damba trypanosome.

Again, the blood of Monkeys 544 and 516, both infected with one and the

same strain of *T. gambiense*, responded regularly and well with different strains of *T. gambiense* trypanosomes. They also, each of them on a few occasions out of many trials, gave a good adhesion reaction with the Damba trypanosome. Monkey 544 was infected by laboratory-bred fly. But Monkey 516, whose blood was just as active in the adhesion tests with both species of trypanosome, was infected direct from man by inoculation of the patient's blood and is therefore free from all suspicion of any contaminating influence. In other words, the observation of a divalent response stands unchallenged and so far unexplained, save by supposing a close relationship between the two trypanosomes.

After a thorough examination of the records of the last few years, I think it is plain that the ruminant reservoir experiments are the only ones in any way open to question. There is no direct evidence of any flaw, but on the other hand it is now no longer possible, save in one instance, to check all the details and produce direct proof to the contrary. The history of Sheep 161 is, however, above suspicion: the animal was still capable of infecting clean *G. palpalis* with *T. gambiense* 15 months after its original infection with that trypanosome. This observation is fully in keeping with the early work with antelope at Mpumu, and goes far to justify the acceptance of the rest of the more recent investigation.

Since February, 1931, the old system has been abandoned and thenceforward every fowl bitten by a possibly infective fly has been numbered and reserved for that experiment only.

Section 2. Evidence furnished by other experiments.

Though not actually devised for this purpose, certain still unpublished experiments carried out to test the effect of diet on trypanosomes developing in the fly have a bearing on the present inquiry.

Paired experiments were conducted under identical conditions of infection (with *T. rhodesiense*), but the two sets of flies were subsequently nourished on different animals. In one series the effects of fowl's and monkey's blood were compared, the birds used being those of the pool. The results were as follows:

In 553 flies nourished on fowl's blood 54 were infected, *i.e.* 9·7 per cent.

In 614 flies nourished on monkey's blood 48 were infected, *i.e.* 7·8 per cent.

In other series the effect of monkey's blood was contrasted with that of the baboon and of *Varanus*. The total figures for all flies nourished on monkey's blood were:

In 978 flies, 85 infected, *i.e.* 8·6 per cent.

At first glance this preponderance of infected flies among those nourished on fowl's blood suggests the possibility of contamination infections from the birds on the pool.

When examined by the formula

$$\chi^2 = \int \frac{(x-m)^2}{m}$$

the figures of the fowl-monkey series give for χ^2 the value 1·400. The result

of the application of the formula to these data suggests, therefore, that the element of chance has an important bearing on this series of tests, and it is possible that these figures could be obtained by chance once in three or four times.

An analysis of the distribution of gut-only and gut-and-gland infections among the two sets of infected flies gives the following figures:

553 nourished on fowls' blood:

Percentage of gut-only infections = 6·8 per cent.
" gut and salivary gland infections = 2·8 "

614 nourished on monkey's blood:

Percentage of gut-only infections = 5·2 per cent.
" gut and salivary gland infections = 2·6 "

The percentage of flies showing salivary gland infections is thus practically identical in the two sets of experiments, what difference there is being confined to the gut-only type of infection.

It should be explained that the flies used in these experiments were carrying *T. rhodesiense*, and that those nourished on fowl's blood were being fed on the birds of the pool at a time when exposure to *T. rhodesiense* was high. To anyone familiar with the transmission of the polymorphic trypanosomes by tsetse a divergence of less than 2 per cent. between two such series possesses no significance; and the correspondence in the figures for the salivary gland infections confirms this impression.

This section thus contributes a further quota of evidence that the fowls of the pool have not interfered with the experiments performed in recent years on the ruminant reservoir of the human trypanosomes. The test is a severe one and would assuredly detect any hidden source of contamination of this nature.

GENERAL CONCLUSIONS.

1. The domestic fowl of Uganda is susceptible to infection with *Trypanosoma rhodesiense* and with *T. brucei* by the bite of *Glossina palpalis* or *G. morsitans*.

2. A fowl so infected with *T. rhodesiense* can infect clean *G. palpalis* fed upon it, but apparently only with very great difficulty. In theory, therefore, the fowl may play a part in the spread of *Trypanosomiasis rhodesiensis* in nature.

3. Of 17 fowls bitten by flies carrying heavy gland infections of *T. rhodesiense* only four gave any evidence of infection. In the case of *T. brucei*, 12 fowls were exposed and only one became infected. Two fowls exposed to the Damba trypanosome failed to become infected.

4. There is some evidence to show that the fowl is only infective to fly in the early stages of its infection, and even then only very slightly. The occurrence of three very slight infections in flies dissected 8 and 10 days after feeding on the infected bird suggests that in the fowl the trypanosome is

usually not in a state favourable to development in the tsetse, although it may so develop in an apparently normal manner.

5. A strain of *T. rhodesiense*, recovered by inoculation into a clean monkey of blood from a fowl experimentally infected by fly-bite, proved in the monkey to be transmissible by tsetse in a perfectly normal manner. Any inhibition of transmissibility exerted by the fowl is therefore temporary and attributable to the influence of the tissues of the bird on the trypanosome.

6. Of the infected fowls observed in the course of the above experiments only one appeared to be ill. This bird showed unilateral conjunctivitis and general malaise. It was killed some 25 days after its infection, and the inoculation of 10 c.c. of its blood infected a clean monkey with *T. rhodesiense* after an incubation period of 6 days. This bird was responsible for one of the two heavy infections found in the 4339 *G. palpalis* dissected in the course of the above experiments.

7. That trypanosomes are very rare in the blood of infected fowls is suggested by the failure to infect a clean monkey by the inoculation of 10 c.c. of the blood of Fowl 39 a day or two after this bird had caused abortive infections in two clean tsetse. For this reason it is difficult to estimate the susceptibility of the fowl to trypanosomes, as blood inoculation is apparently not an infallible method of demonstrating infection.

8. From the experiments described in this paper it appears very unlikely that the fowl need be regarded as a factor in the spread of human trypanosomiasis in nature, at all events in *G. palpalis* regions.

9. There is evidence that the fowl may be more susceptible to infection with *T. rhodesiense* than with *T. brucei*, but only one strain of *T. brucei* was tested.

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I wish to record my indebtedness to my colleague, Dr J. M. Wallace, who has undertaken all the sub-inoculations of fowls' blood and rendered general assistance in carrying out the experimental section. Also to Mr J. S. McDonald, Laboratory Assistant, for his efficient supervision of the animals used in these experiments.

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REPORT ON A COLLECTION OF PARASITIC NEMATODES, MAINLY FROM NORTH CHINA.

PART I. FILARIOIDEA.

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(With 53 Figures in the Text.)

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Introduction.

The parasitic nematodes described in this paper were collected by Dr E. C. Faust during his stay in China, and sent to Prof. Hoeppli for identification and study. The total collection amounts to about 500 vials, the majority of which were collected in Peiping (Peking), mainly from birds.

Under Prof. Hoeppli's direction the writer has had the great pleasure of studying this large collection. The present paper is a first instalment of a report on the nematode parasites, and covers the description of eight species of *Diplotriaena*, with a discussion of their systematic characters: *D. tricuspis* (Fedtschenko 1874) Seurat 1915, *D. unguiculata* Rudolphi 1819, *D. alaudae* Zeder 1803, *D. nochti* Hoeppli and Hsü 1929, *D. tridens* (Molin 1858) Boulenger 1928, *D. microphallos* n.sp., *D. diucae* Boulenger 1920, *D. cantonensis* n.sp.

and six other filariid worms; *Onchocerca bambusicolae* n.sp., *Serratospiculum turkestanicum* Skrjabin 1916, *Hamatospiculum brasilianum* Skrjabin 1916; *Pseudaprocta decorata* n.sp., *Chandlerella sinensis* n.sp., and *Setaria equina* (Abildgaard 1789) Railliet and Henry 1911.

GENUS *DIPLOTRIAENA* RAILLIET AND HENRY 1909.

This genus was established by Railliet and Henry in 1909 to include a group of closely allied species, the majority of which have been incompletely described. Since then the number of species of this genus has greatly increased, until at present, in addition to the twenty species listed by Yorke and Maplestone (1926), ten new species have been described: *D. multituberculata* Walton 1928, *D. americana* Walton 1928, *D. tridentis* Walton 1928; *D. nochti* Hoeppli and Hsü 1929; *D. corrugata* Wehr 1930; *D. manipoli* Chu 1931; *D. bifidus* Canavan 1931; *D. graculi*, *D. dubia* and *D. urocissae* Maplestone 1931; and three old species have been revived: *D. obtusocaudata* (Rudolphi 1819) by Walton in 1928, and *D. tridens* (Molin 1858) and *D. monticelliana* (Stossich 1890) by Boulenger in 1928.

Consequently there are at least thirty described species to be dealt with in this genus, all of which are from birds. The old descriptions of the majority of the species are so incomplete as to make their identification extremely difficult. Furthermore, the systematic characters used for distinguishing the different species are known to be notoriously difficult to make out. As a result several species have been confused into one, such as in the case of *D. tricuspis*, which, judging from the great variation of body length (male, 25–65 mm.; female, 50–165 mm.), and the large number of hosts as recorded in *Filarie e Spiroptere* (Stossich, 1896), will, on re-examination of the original material, undoubtedly prove to be composed of several different species. On the other hand it is also highly probable that there are cases in which the same species has been differently described by different writers.

A thorough revision of this genus as a whole has not yet been carried out, and until such is done the diagnosis of *Diplotriaena* species, in most cases, can only be regarded as provisional. In the present paper, in addition to describing eight species of *Diplotriaena*, the writer has made an attempt to bring out certain characters, which may be of taxonomical interest for the study of the genus as a whole.

Discussion of the systematic characters.

Oesophagus. The oesophagus of the species of *Diplotriaena* has been described as either simple or divided into an anterior muscular and a posterior glandular portion. In a study of histological sections of these two types of oesophagus we have found that, in reality, both are divided. The anterior portions, reaching a little behind the nerve ring, are in both cases muscular; the posterior part of the oesophagus in the so-called divided type is surrounded by the oesophageal glands forming a more or less triangular mass in cross-

section; in the so-called simple type the corresponding portion of the oesophagus is also surrounded by the glands, but in the form of a very delicate branching structure. These branches are so thin that in the unstained specimen they do not show up well, and therefore usually only the central portion of the oesophagus has been noticed and described as simple and slender. Although fundamentally these two types of oesophagus are both divided, yet the difference in their gross appearance is so obvious that this character of the oesophagus still provides a convenient means of subdividing the whole genus into two main groups so as to facilitate our taxonomic study.

Cephalic papillae. We have observed, on the anterior end of all eight species of *Diplotriaena*, two rows of sub-medial papillae, four each in number. Those of the outer row are the larger, while those of the inner row are the smaller and sometimes difficult to see (Fig. 28). With the exception of three species (*Chandlerella sinensis* n.sp., *Pseudaprocta decorata* n.sp. and *Onchocerca bambusicolae* n.sp.), this observation also holds true for all the other filariid worms studied in this paper. In the case of *Setaria equina*, the head papillae of the inner row are modified into the form of spines.

Striations. The presence or absence of transverse striations in the cuticle is one of the characters commonly used for distinguishing different species. The writer is of the opinion that we should be more careful in making use of this character, for on the one hand the striation of the cuticle, as usually found in the filariid worms, is so delicate, that it can only be detected by careful searching under high magnification, while on the other hand wrinkles and folds which are purely artefacts of fixation, may be mistaken for striations. The cuticle of filariae is usually very thin, so that artefacts are frequently produced from poor fixation. In our experience with *Diplotriaena*, when the worms were fixed directly after being taken out from the host, the cuticle of a well-straightened worm presented a smooth appearance. When the worm was left in normal saline, even for a few minutes before fixation, the cuticle immediately became swollen, and wrinkles and folds appeared on it. When a living specimen was observed under the microscope, the wrinkles, especially on the curved portion of the worm, looked very much like the striations of the cuticle; but these disappeared when the worm was straightened or bent to the other side. In all eight species of *Diplotriaena* studied, we found wrinkles and folds on the cuticle, but no striations.

Tridents. The pair of tridents is a conspicuous character of the genus, and yet a comparative study of these in the different species has, so far as we can ascertain, not yet been done. The writer has made an attempt to draw the tridents (one representing each specimen) of the different species in our possession, in order to find out to what extent they can be used as a systematic character. The result is shown in Figs. 46–53, a study of which will show the following points:

(1) Within the same species, with very few exceptions, the tridents conform very well both in shape and in size.

(2) Among different species, similar tridents as well as differing ones were found.

(3) In spite of the great difference of body length between males and females, there was practically no sexual difference in the tridents either in shape or in size.

The characters of the tridents of different species are, therefore, of a certain taxonomic value, especially when they are used in combination with other characters.

In *D. corrugata* Wehr (1930) described a corrugated appearance of the tridents. We have observed a similar corrugation in all species of our worms except in *D. microphallos* and *D. tridens*, of which we have only single specimens which have already turned dark brown. The corrugation, however, is especially conspicuous in *D. nochti*, *D. diucae* and *D. cantonensis*.

The function of the tridents still remains a problem. In our fixed specimens they were found either protruded or retracted from the anterior end. From their chitin-like nature and from their position, we are inclined to assume that they are structures for boring or attachment, since chitin-like structures probably for similar purpose have been frequently met with in various forms on the anterior end of other filariid worms.

When a living specimen of *D. diucae* was observed in normal saline under a cover glass, the pair of tridents could be seen moving in and out. The movement became much more vigorous when the anterior end came into contact with a mass of blood cells, which gave a certain resistance; the tips of the tridents were then found to protrude definitely outside the cuticle. In the fixed specimens the mouth is a narrow slit; but when a living specimen is in the act of ingestion, a pair of small lips becomes visible at the mouth (Fig. 11).

Male caudal papillae. The number and arrangement of caudal papillae in the male, which is a very useful character in determining species of other genera, do not turn out to be so useful in *Diplotriaena*. As described by various writers they vary from none to thirteen pairs in the different species. In view of the difficulty experienced in distinguishing these papillae, we agree with Boulenger (1920) that not much reliance can be placed upon them. Most of these papillae are extremely small and only slightly prominent, and some are situated along the margin of the posterior extremity, so that in a ventral mount, if slightly pressed, they may become invisible by moving to the other side. Our findings with regard to the male papillae agree with the observations made by Boulenger in 1928 and Hoeppli and Hsü in 1929. In all eight species of *Diplotriaena* studied, we found that there are three groups of papillae. The drawing of *D. nochti* Hoeppli and Hsü, 1929, illustrates precisely the arrangement of these three groups. In the first group there are four to five pairs of papillae arranged in two oblique rows, one on each side of the anus; the last one or two of the series may be ad-anal or post-anal, while the rest are pre-anal in position. In the second group the number varies from one to three which are post-anal and ventral in position. In the third group there are

usually three pairs of papillae situated along the margin of the posterior extremity and more lateral in position.

Spicules. The shape of the two spicules is characteristic for the genus. The left spicule is shaped in the form of a long needle slightly curved and varies considerably in length in the different species. The right spicule is shorter and spiral shaped. The number of turns of the spiral spicule as described by various writers for the different species varies from one to six. On close comparison we found that it is rather difficult to determine exactly the number of turns of this spiral. The fundamental structure of the small spicule is practically the same in all the species studied in this paper. It consists of a flattened spiral tube with its lateral margins more or less coalesced; the difference in degree of coalescence in the different species is revealed, in the gross appearance, by the presence or absence of alae on the spiral spicule. We also found spiral spicules provided either with one ala on each side, on only one side, or none at all. This point can also be used as a systematic character. The illustrations of the cross-sections of the small spicules made in the middle of each one will serve to make this point clear.

Vulva. The vulva of all the species is situated in the oesophageal region. The relatively small difference among the different species together with the considerable variation within the same species, renders the position of the vulva of little use as a systematic character to distinguish the different species.

Uteri. With the exception of *D. unguiculata* the arrangement of the two uteri in all the females is similar: the two uteri run parallel caudad for a considerable distance, usually passing the middle of the body, when one turns anteriad with its ovary extending to the oesophageal region; while the other continues its course to the posterior end. In the case of *D. unguiculata* the two uteri are practically parallel in their whole course. They run backward to the posterior end, where, at a short distance from each other, both of them turn forward giving the appearance of two pairs of uterine tubes along their course.

Excretory pore. The position of the excretory pore is slightly posterior to the nerve ring in all the species.

Diplotriaena tricuspis (Fedtschenko 1874) Seurat 1915.

(Figs. 10, 13, 13 A and 47.)

Hosts: (1) *Corvus sinensis*, (2) *C. neglectus*, (3) *C. torquatus*, (4) *C. corax.*
Location: Mesentery.
Locality: Peiping, China.
Material: Numerous.
Specimens: Helminth. Coll.[1], Pe. Nos. (1) 1175C, (2) 571B, (3) 572, (4) 307.

[1] Signifying throughout this paper the collection at Peiping Union Medical College, Peiping, China.

Male:	Number of specimens measured	6
	Body length	48·00– 56·00[1]
	Body width	0·65– 0·68
	Nerve ring to anterior end	0·23– 0·25
	Trident	0·10– 0·12
	Oesophagus: Muscular portion	0·35– 0·39
	Glandular portion	4·00– 4·45
	Spicules: Left	1·37– 1·54
	Right	0·86– 0·92
Female:	Number of specimens measured	5
	Body length	140·00–152·00
	Body width	1·05– 1·28
	Nerve ring to anterior end	0·20– 0·22
	Trident	0·09– 0·12
	Oesophagus: Muscular portion	0·28– 0·35
	Glandular portion	4·28– 4·70
	Vulva to anterior end	0·75– 0·80
	Egg	0·03–0·04 × 0·05– 0·06

Anterior end with four pairs of sub-medial papillae and the amphids. Oesophagus of the divided type. Tridents very short; cuticle smooth. Ten pairs of caudal papillae typically arranged in the male (Seurat described six pairs of caudal papillae, four pre-anal and two post-anal). Short spicule with one ala and twisted spirally for about one and half turns.

The confusion of several species under this name has already been discussed. In 1915 Seurat redescribed worms collected from *Corvus corax* as *D. tricuspis*. More recently Boulenger (1928) also referred worms from *Corvus cornix* and *C. umbrinus* to the same species. Our specimens collected from *C. sinensis*, *C. neglectus*, *C. torquatus*, and *C. corax* agree very closely with the description given by Seurat, especially in the small size of the trident and the length of the long and short spicules. We have, therefore, referred our specimens to the same species as that described by Seurat. The measurements and a brief description are given for comparison.

Diplotriaena unguiculata Rudolphi 1819.

(Figs. 23, 26, 26 A, 35 and 48.)

Revised diagnosis. *Diplotriaena*: very closely related to *D. tricuspis* but differing in the shape and size of the tridents, in the short spicule which possesses two alae instead of one, and in the arrangement of the uteri and ovaries.

Host: *Alauda arvensis*.
Location: Mesentery.
Locality: Peiping, China.
Material: Four males and one female.
Specimens: Helminth. Coll., Pe. No. 565 X.

[1] All dimensions in mm. unless otherwise stated.

Male:	Number of specimens measured	4
	Body length	56·00– 66·00
	Body width	0·48– 0·55
	Nerve ring to anterior end	0·25– 0·28
	Trident	0·12– 0·15
	Oesophagus: Muscular portion	0·72– 0·75
	Glandular portion	4·00– 4·40
	Spicules: Left	1·15– 1·22
	Right	0·72– 0·75
Female:	Number of specimens measured	1
	Body length	158·00
	Body width	0·70
	Nerve ring to anterior end	0·25
	Trident	0·12– 0·14
	Oesophagus: Muscular portion	0·51
	Glandular portion	5·10
	Vulva to anterior end	0·82
	Eggs	0·03 × 0·05

Oesophagus of the divided type. Mouth surrounded by four pairs of submedial papillae and the amphids. Cuticle smooth.

Male. Eleven pairs of caudal papillae typically arranged. Spicules relatively long; the short spicule with two alae and twisted spirally in about one and half turns.

Female. Vulva slightly prominent, situated near the beginning of the glandular portion of oesophagus. Uteri parallel: both uterine tubes run backward to the posterior end, where, at a short distance from each other, both of them turn forward giving the appearance of four uteri along their courses.

From *Alauda arvensis* we found two species of *Diplotriaena*. From the same host, Zeder described *Filaria alaudae* in 1803 and Rudolphi *F. unguiculata* in 1819. In Stossich's Monograph of Filariids (1896), he listed both of these species as synonyms of *D. tricuspis*. Since a number of species have been confused under this name, it is highly probable that *F. alaudae* and *F. unguiculata* are distinct species and not synonyms of *D. tricuspis*. From the papers by Zeder (1803), Rudolphi (1819), Dujardin (1845), Diesing (1851) and Molin (1858), we cannot discover any indication of the presence of tridents or of the spiral spicule, which are the essential characters of the genus. But if Stossich is correct in his diagnosis of the genus, it is very likely that they may be the same species now studied by us.

Besides the identity of hosts, there is also an approximate agreement of body length in each of the two species. We, therefore, have referred our species to *D. alaudae* and *D. unguiculata*, in spite of the inadequacy of the original description, and at the same time we give their revised diagnosis for comparison with other species.

Diplotriaena alaudae Zeder 1803.

(Figs. 22, 25, 25 A, 36 and 51.)

Revised diagnosis. Diplotriaena: very similar to *D. diucae* but different from it in having much larger tridents and eleven pairs of caudal papillae in the male with typical arrangement.

Host: *Alauda arvensis.*
Location: Mesentery.
Locality: Peiping, China.
Material: One male and five females.
Specimens: Helminth. Coll., Pe. No. 565 A.

Male:	Number of specimens measured	1
	Body length	35·00
	Body width	0·55
	Nerve ring to anterior end	0·23
	Trident	1·75
	Oesophagus	4·10
	Spicules: Left	0·60
	Right	0·47
Female:	Number of specimens measured	5
	Body length	53·00–67·00
	Body width	0·51– 0·65
	Nerve ring to anterior end	0·25– 0·28
	Trident	0·16– 0·20
	Oesophagus	4·00– 4·30
	Vulva to anterior end	0·37– 0·50
	Eggs	0·01–0·013 × 0·025– 0·03

Oesophagus of the simple type; mouth surrounded by four pairs of submedial papillae and the amphids. Cuticle smooth. Tridents rather long, corrugation only occasionally found.

Male. Nine pairs of caudal papillae typically arranged. Spicules relatively short; short spicule slender without ala and twisted in about half a turn.

Female. Ovary divergent; eggs thin shelled and comparatively small.

Diplotriaena nochti Hoeppli and Hsü 1929.

(Figs. 12, 12 A, 14 and 46.)

Host: *Spodiospar cineraceus.*
Location: Mesentery.
Locality: Peiping, China.
Material: Numerous.
Specimens: Helminth. Coll., Pe. Nos. 560 A, 518 A, 592 A, 1172, 544, 558 B, 592 D.

Male:	Number of specimens measured	5
	Body length	38·00– 50·00
	Body width	0·55– 0·60
	Nerve ring to anterior end	0·18– 0·24
	Trident	0·15– 0·16
	Oesophagus: Muscular portion	0·27– 0·44
	Glandular portion	6·80– 7·90
	Spicules: Left	2·40– 2·80
	Right	0·57– 0·65

Female: Number of specimens measured	... ...	5
Body length	... ...	110·00–156·00
Body width	... ...	0·85– 0·99
Nerve ring to anterior end	... ...	0·21– 0·31
Trident	... ...	0·13– 0·15
Oesophagus: Muscular portion	... ...	0·26– 0·31
Glandular portion	... ...	9·62– 9·72
Vulva to anterior end	... ...	0·65– 0·70
Eggs	... ...	0·030–0·035 × 0·040– 0·052

This species is easily distinguished from the others by the great length of the long spicule and of the glandular portion of the oesophagus. Cuticle smooth; anterior end with four pairs of sub-medial papillae and the amphids. Short spicule with two alae and twisted spirally in little more than one turn. Tridents usually with corrugation on all three arms. Eleven pairs of caudal papillae arranged as shown in the original description.

Diplotriaena tridens (Molin 1858) Boulenger 1928.

(Figs. 15, 18 and 50.)

Host: *Lanius superciliosus*.
Location: Mesentery.
Locality: Peiping, China.
Material: One male.
Specimens: Helminth. Coll., Pe. No. 839A.

Male: Number of specimens measured	... ...	1
Body length	... ...	27·50
Body width	... ...	0·59
Nerve ring to anterior end	... ...	0·30
Trident	... ...	0·17
Oesophagus: Muscular portion	... ...	0·40
Glandular portion	... ...	4·00
Spicules: Left	... ...	0·68
Right	... ...	0·46

The original description given by Molin is very incomplete. Boulenger in 1928 referred a small form of *Diplotriaena* from a related host (*Lanius collurio*) to this species. Our material agrees fairly well with the description as given by Boulenger; the measurements of our worm are slightly greater with a corresponding increase of the body length.

Oesophagus divided; tridents rather long; cuticle smooth. Six pairs of caudal papillae observed (Boulenger described eight to ten pairs of caudal papillae). Short spicule with one ala and twisted spirally in little more than one turn.

Diplotriaena microphallos n.sp.

(Figs. 17, 20 and 49.)

Specific diagnosis. *Diplotriaena*: A relatively great body length with relatively very short spicules, especially the left one.

Host: *Anas boschas*.
Location: Mesentery.

Locality: Peiping, China.
Material: One male.
Specimens: Helminth. Coll., Pe. No. 71C.

Male:	Number of specimens measured	1
	Body length	51·50
	Body width	0·92
	Nerve ring to anterior end	0·27
	Trident	0·12
	Oesophagus: Muscular portion	0·30
	Glandular portion	3·40
	Spicules: Left	0·57
	Right	0·47

Oesophagus of the divided type; cuticle smooth. Seven pairs of caudal papillae observed. Spicules relatively very short especially the left one; the right spicule is provided with one ala and twisted in about one and half turns.

Filaria anatis has been recorded from *Anas boschas*. But from papers by Rudolphi (1809), Dujardin (1845), Diesing (1851) and Molin (1858), we cannot discover any indication for a *Diplotriaena*. The combined character of a fairly great body length with very short spicules of our worm, does not fit into any of the described species. So in spite of having only a single male specimen as a basis, we believe that it is better to regard our material as a new species rather than to further complicate the inadequately described species of this genus.

D. dubia recently described by Maplestone (1931), was given a body length of 8·55 mm.; but in the description it was mentioned as "about twice the length of the male of *D. gracilis*," which has a body length of 27·5–32 mm. According to the latter statement, *D. dubia* is about the same length as the present species. Our species still differs from *D. dubia*, however, in having shorter spicules and a much longer oesophagus, more than twice as long as that of *D. dubia*.

Diplotriaena diucae Boulenger 1920.

(Figs. 16, 19, 19 A, 38 and 52.)

Hosts: (1) *Emperiza cioides*, (2) *E. schoeniclus*, (3) *E. leucocephala*, (4) *E. pusilla*.
Location: (1) mesentery, (2) lung and trachea, (3) mesentery, (4) left lung.
Locality: Peiping, China.
Material: Numerous.
Specimens: Helminth. Coll., Pe. Nos. (1) 570B, 600B, 604, (2) 34A, (3) 574B, (4) 27.

Male:	Number of specimens measured	4
	Body length	25·00–29·00
	Body width	0·65– 0·70
	Nerve ring to anterior end	0·23– 0·24
	Trident	0·12– 0·13
	Oesophagus	3·8– 3·85
	Spicules: Left	0·70– 0·77
	Right	0·47– 0·55

Female:	Number of specimens measured	5
	Body length	44·00–48·00
	Body width	0·75– 0·80
	Nerve ring to anterior end	0·24– 0·26
	Trident	0·12– 0·14
	Oesophagus	4·30– 4·50
	Vulva to anterior end	0·45– 0·48
	Eggs	0·02–0·03 × 0·04– 0·05

Our observations on the present material agree very closely with those given by Boulenger for *D. diucae* (1920). They agree particularly well in the shape and size of tridents and spicules.

Oesophagus of the simple type. Anterior end with four pairs of sub-medial papillae and the amphids. Tridents comparatively small, usually with conspicuous corrugations. Cuticle smooth. Ten pairs of caudal papillae observed (Boulenger observed six pairs of caudal papillae, four pre-anal, and two post-anal). Short spicule without ala and twisted spirally in only half a turn.

Diplotriaena cantonensis n.sp.

(Figs. 21, 24, 24 A and 53.)

Specific diagnosis. Diplotriaena: Arms of tridents close together with conspicuous corrugations. Oesophagus of the simple type. Spicules very short; the short one with one ala and twisted in about one turn.

Host: *Otocompsa jocosa jocosa* Linn.
Location: Body cavity.
Locality: Canton, China.
Material: Three males and one female.
Specimens: Helminth. Coll., Can. No. 5.

Male:	Number of specimens measured	3
	Body length	22·00–23·00
	Body width	0·50– 0·55
	Nerve ring to anterior end	0·25– 0·26
	Trident	0·14– 0·15
	Oesophagus	3·15– 3·20
	Spicules: Left	0·65– 0·66
	Right	0·46– 0·48
Female:	Number of specimens measured	1
	Body length	39·00
	Body width	0·65
	Nerve ring to anterior end	0·30
	Trident	0·17
	Oesophagus	3·65
	Vulva to anterior end	0·58
	Eggs	0·02–0·03 × 0·04– 0·05

Mouth surrounded by four pairs of sub-medial papillae and the amphids. Oesophagus of the simple type. Cuticle smooth. Arms of the trident very close together; corrugation very conspicuous and usually found in all three arms.

Male: Seven pairs of caudal papillae observed. Spicules relatively short; short spicule with one ala and twisted in little more than one turn.

Female: Vulva prominent; ovaries opposite; one extends to the level of the vulva, while the other nearly reaches the posterior end. Eggs oval, thick shelled containing well-developed embryos.

D. urocissae, recently described by Maplestone (1931) possesses about the same body length and trident length as the present species, which, however, differs from the former in the characters of the short and the long spicules and also in the length of the oesophagus.

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Onchocerca bambusicolae n.sp.

(Figs. 29, 31, 33, 34, 37 and 39.)

Specific diagnosis. Onchocerca: Cuticle in both sexes very finely striated transversely and reinforced externally by circular thickenings; there are in addition rod-like cuticular thickenings at the ventral surface of the coiled part of the posterior end of the male.

Host: *Bambusicola thoracica.*
Location: Body cavity.
Locality: Peiping, China.
Material: Two males and one female.
Specimens: Helminth. Coll., Pe. No. 739 B.

Male:	Number of specimens measured	2
	Body length	31·00–35·00
	Body width	0·25– 0·35
	Nerve ring to anterior end	0·22– 0·22
	Excretory pore to anterior end	0·27
	Oesophagus: Muscular portion	0·43– 0·45
	Glandular portion	3·85– 4·00
	Spicules: Left	0·19– 0·21
	Right	0·14– 0·15
	Tail	0·13– 0·15
	Width of annular thickenings	0·002
	Distance between annular thickenings	0·012– 0·018

Female: Number of specimens measured	1
Body length	53·00
Body width	0·70
Nerve ring to anterior end	0·17
Excretory pore to anterior end	0·27
Oesophagus: Muscular portion	0·50
Glandular portion	5·86
Vulva to anterior end	4·00
Tail	0·12
Width of annular thickenings	0·002
Distance between annular thickenings	0·015– 0·02

Both extremities strongly attenuated, especially the anterior end of the female. Mouth simple, surrounded by the amphids and two pairs of submedial papillae. Cuticle with very delicate, transverse striations and reinforced externally by annular thickenings. Oesophagus divided into two parts: the anterior portion being muscular and relatively short, while the posterior portion is glandular and long. Intestine very narrow.

Male. Tail spirally coiled. Caudal alae absent. Four pairs of ad-anal papillae. Spicules unequal and dissimilar, the left one being the longer, and shaped like a surgical needle. Gubernaculum absent. At the ventral surface of the coiled part of the posterior end there are rod-like cuticular thickenings arranged in transverse rows. Caudal pores near posterior end.

Female. Vulva situated at middle of the glandular portion of the oesophagus. Opisthodelphys: one ovary ends near the posterior end with its seminal receptacle at 9·1 mm. from it; while the other ends more anteriorly with its seminal receptacle at 22·8 mm. from the posterior end. Viviparous: eggs of all stages and fully developed larvae were found in the uteri. Anus very small and somewhat atrophied. Caudal pores near posterior end.

In addition to the genus *Onchocerca* the sub-family *Onchocercinae* contains two other genera: *Elaeophora* and *Katanga*. The present species resembles *Elaephora poeli* in the characters of the caudal papillae and the spicules of the male as figured in Yorke and Maplestones's textbook; but in *E. poeli* the cuticle is described as exhibiting internal striation, while in our specimen it shows external annular thickening. Moreover our female specimen, although showing a marked attenuation of the anterior end, is not posteriorly thickened so greatly as described for *E. poeli*.

This species also resembles *Katanga katangensis* in having additional rod-form cuticular thickenings. The cuticular thickenings in *K. katangensis*, however, are irregularly distributed in both males and females, while in our material they are confined to the ventral surface of the coiled part of the posterior end of the male and are also arranged in transverse rows.

So far as we can find out from the literature, this is the first record of an *Onchocerca* from birds.

Literature to foregoing section: BERNARD, P. N. and BAUCHE, J. (1912). *Bull. Soc. Path. Exot.* **5**, 109.—BRUMPT, E. (1919). *Ibid.* **12**, 464.—CASTELLANI, A. (1925). *J. Trop. Med. and Hyg.* **28**, 1.—COMMES, CH. and DEVANELLE, P. (1917). *Bull. Soc. Path. Exot.* **10**, 459.—FÜLLEBORN, F. (1908). *Arch. f. Schiffs- u. Tropenhyg.* **12**, Beiheft 7, 291.—GILRUTH, J. A. and SWEET, G. (1912). *Proc. Roy. Soc. Victoria*, **25**, n.s., 23.—HOEPPLI, R. and HSÜ, H. F. (1929). *Arch. f. Schiffs- u. Tropenhyg.* **33**, Beiheft 1, 33.—LEIPER, R. T. (1911 *a*). *J. Lond. Sch. Trop. Med.* **1**, 22; (1911 *b*). *Rept. Local Govt. Board on Publ. Health and Med. Subjects*, n.s., No. 45, p. 6; and *J. Trop. Med. and Hyg.* **14**, 87.—PADER, J. (1901). *Arch. Parasitol.* **4**, 58.—RAILLIET, A. and HENRY, A. (1910). *C.R. Soc. Biol.* **68**, 248; (1912). *Bull. Soc. Path. Exot.* **5**, 115.

Serratospiculum turkestanicum Skrjabin 1916.

(Figs. 40, 41 and 45.)

Host: *Hypotriorchis aesalon*.
Location: Body cavity.
Locality: Peiping, China.
Material: Two males and two females.
Specimens: Helminth. Coll., Pe. No. 318.

Male:	Number of specimens measured	2
	Body length	95 –100
	Body width	0·38– 0·40
	Nerve ring to anterior end	0·19– 0·20
	Oesophagus: Muscular portion	0·47– 0·50
	Glandular portion	6·90– 7·60
	Spicules: Left	0·50– 0·70
	Right	Incomplete
	Tail	0·07– 0·08
Female:	Number of specimens measured	2
	Body length	120 –140
	Body width	0·40– 0·45
	Nerve ring to anterior end	0·22– 0·23
	Oesophagus: Muscular portion	0·55– 0·60
	Glandular portion	6·85– 8·70
	Vulva to anterior end	0·65– 0·90
	Eggs	0·020–0·025 × 0·04– 0·05
	Tail	0·04– 0·05

There are some differences between our material and *S. turkestanicum* as described by Skrjabin (1916). In his description he mentions that there is no transverse striation in the cuticle and that there are six pairs of pre-anal papillae. In our material we found in the cuticle extremely delicate, transverse striations and only five pairs of post-anal papillae; a structure which resembles a sixth pair of the post-anal papillae we regard as caudal openings. The eggs of our specimens are also smaller. Nevertheless we record our material as *S. turkestanicum* because it agrees in all other important characters and also in measurements. If in the future a re-examination should show that these differences are not due to an oversight or to variations, a new species may then be created for these specimens.

The distal ends of the short spicules of both of our male specimens were

unfortunately broken off, so we were not able to compare the characters on these structures. The small spicule in our specimen is apparently dislocated. The cuticular bosses are few in the males, but numerous in the females.

Literature to foregoing section: BAYLIS, H. A. (1925). *Ann. Mag. Nat. Hist.*, ser. 9, **15**, 112.—HOEPPLI, R. and HSÜ, H. F. (1929). *Arch. f. Schiffs- u. Tropenhyg.* **33**, Beiheft 1, 31.—MOLIN, R. (1858). *Sitzungsber. k. Akad. Wiss. Wien (math.-naturw. Cl.)*, **28**, 365.—RAILLIET, A. and HENRY, A. (1916). *Bull. Soc. Path. Exot.* **9**, 364.—SCHNEIDER, A. (1866). *Monographie der Nematoden*, Berlin.—SEURAT, L. G. (1915). *Novitates Zoologicae*, **22**, 1.—SKRJABIN, K. J. (1916). *Ann. Mus. Zool. Acad. Impériale Sci. Pétrograd*, **20**, 457; (1917). *Parasitology*, **9**, 460.

Hamatospiculum brasilianum Skrjabin 1916.

(Figs. 42 and 44.)

Synonyms: *Filaria brasilianum* Stoss. 1897.
Filaria insignis Schneider 1866.
Host: *Lanius sphonocercus*.
Location: Small intestine? (probably mesentery).
Locality: Peiping, China.
Material: One male and the anterior portion of a female.
Specimens: Helminth. Coll., Pe. No. 285 A.

Male:	Number of specimens measured	1
	Body length	23·00
	Body width	0·55
	Nerve ring to anterior end	0·16
	Oesophagus: Muscular portion	0·35
	Glandular portion	6·10
	Spicules: Left	Incomplete
	Right	0·32
	Tail	0·06
Female:	Number of specimens measured	1
	Body length	Incomplete
	Body width	0·70
	Nerve ring to anterior end	0·16
	Excretory pore to anterior end	0·17
	Oesophagus: Muscular portion	0·40
	Glandular portion	6·70
	Vulva to anterior end	1·00
	Eggs	0·03 × 0·05

Only one male and a fragment of the anterior end of a female were available for study. The main characters and measurements, so far as we could make out from our material, agree with those given by Skrjabin (1916), with the exception that in Skrjabin's drawing there appears to be one more pair of post-anal papillae. Owing to the incompleteness of our specimens, a more thorough study was not possible.

Literature to foregoing section: SCHNEIDER, A. (1866). *Monographie der Nematoden*, Berlin.—STOSSICH, M. (1897). *Boll. Soc. adriat. di sc. nat. in Trieste*, **18**, 43.—YORKE, W. and MAPLESTONE, P. A. (1926). The *Nematode Parasites of Vertebrates*, p. 395.

Setaria equina (Abildgaard 1789) Railliet and Henry 1911.

(Fig. 27.)

Host: *Equus caballus*.
Location: Mesentery.
Locality: Peiping, China.
Material: Three females.
Specimens: Helminth. Coll., Pe. No. 1211 B.

Female: Number of specimens measured	3
Body length	87·00–125·00
Body width	0·80– 0·90
Nerve ring to anterior end	0·25– 0·26
Excretory pore to anterior end	0·28
Cervical papilla to anterior end	0·67
Oesophagus: Muscular portion	1·00– 1·15
Glandular portion	10·50– 11·40
Vulva to anterior end	0·70– 0·80
Eggs	0·015–0·02 × 0·02– 0·03
Tail	0·40– 0·60

Illustrations of this well-known species were only recently given by Yorke and Maplestone (1926), and Boulenger (1928). Our specimens consist of three females only and they agree very well in their main characters with those given by these authors.

The mouth is surrounded by a chitinous ring which protrudes anteriorly into four lips. The female tail is bent into a spiral with a pair of lateral cuticular appendages near the tip. A short section of the cuticle at the anterior end shows a striated appearance, while the rest is all smooth.

One point, however, should be emphasised that in the anterior end, as found in the majority of filariid worms described in this paper, there are also two rows of papillae, the inner row of which is modified into spinous processes.

Literature to foregoing section: BOULENGER, C. L. (1928). *Parasitology*, **20**, 36.—RAILLIET, A. and HENRY, A. (1911). *Bull. Soc. Path. Exot.* **4**, 386.—YORKE, W. A. and MAPLESTONE, P. A. (1926). *The Nematode Parasites of Vertebrates*, p. 422.

Pseudaprocta decorata n.sp.

(Figs. 1, 3, 5 and 9.)

Specific diagnosis. *Pseudaprocta*: Very closely related to *P. gubernacularia* Shikhobalov 1930, but differentiated from it by the possession of narrow caudal alae, and a larger number of pre-anal and ad-anal papillae in the male.

Hosts: (1) *Corvus corax*, (2) *Pica caudata*.
Locations: (1) thoracic cavity, (2) small intestine? (probably mesentery).
Locality: Peiping, China.
Material: Numerous.
Specimens: Helminth. Coll., Pe. Nos. (1) 51 D, (2) 265 B.

Male:	Number of specimens measured	3
	Body length	16·00–18·00
	Body width	0·34– 0·42
	Nerve ring to anterior end	0·13– 0·14
	Cervical papillae to anterior end	0·29– 0·33
	Oesophagus	0·58– 0·68
	Spicules	0·35– 0·39
	Gubernaculum	0·06– 0·08
	Tail	0·18– 0·20
Female:	Number of specimens measured	4
	Body length	30·00–32·00
	Body width	0·51– 0·65
	Nerve ring to anterior end	0·14– 0·16
	Excretory pore to anterior end	0·21– 0·32
	Oesophagus	0·68– 0·76
	Vulva to anterior end	0·34– 0·46
	Eggs	0·020–0·023 × 0·03– 0·05
	Tail	0·13– 0·17

Anterior end armed with extremely minute spines arranged in a radiating pattern between the mouth opening and the head sense organs. A short mouth cavity present; mouth simple surrounded by the amphids, and two pairs of sub-medial papillae; cuticle with very fine, transverse striations; oesophagus slightly enlarged posteriad and not divided into two parts. Nerve ring at the beginning of the second fourth of oesophagus.

Male. Tail slightly curved ventrad. Caudal alae narrow. Ten pairs of caudal papillae: six pre-anal, two ad-anal and two post-anal, with an unpaired small papilla just in front of the anus (in one case this papilla was absent). The arrangement of the ad-anal papillae and of some of the pre-anal ones is either symmetrical or asymmetrical. Spicules sub-equal, sub-similar, the left one being more slender and approaching the shape of L more than the right one. A small short gubernaculum present.

Female. Vulva at oesophageal region. Both uteri running posteriad until slightly anterior to the middle of body, where one of them continues its course posteriad, while the other turns anteriad with its ovary extending to the oesophageal region. Eggs small and oval.

The present species is very closely related to *P. gubernacularia* as described by Shikhobalov in 1930, except in the possession of the caudal alae, narrow but distinct, and a larger number of pre-anal and ad-anal papillae. The measurements of the male as given by this author are practically the same as those of our specimens, while the measurements of the females are much smaller, especially in the body length. Since Shikhobalov's material consists of only one male and three females, which are without larvae or eggs, it seems probable that our differences may be due to immature conditions or incomplete observations on account of the scarcity of Shikhobalov's material. Yet, until the re-examination of the type specimens from Russia, we consider it preferable to create a new species for the present material on account of the four additional pairs of caudal papillae and the presence of the caudal alae.

Literature to foregoing section: DRASCHE, R. (1882–3). *Verhandl. d. k. k. zool.-bot. Gesellsch. Wien* (1881), **32**, 117; **33**, 107, 193.—MOLIN, R. (1860). *Sitzungsber. d. k. Akad. Wiss. Wien (Math.-naturw. Cl.)*, **38**, 911.—RAILLIET, A. and HENRY A. (1910). *C.R. Soc. Biol.* **68**, 248.—SHIKHOBALOV, N. (1930). *Ann. Parasitol.* **8**, 624.

Chandlerella sinensis n.sp.

(Figs. 2, 4, 7 and 8.)

Specific diagnosis. Chandlerella: Closely related to *C. bosei*, but distinguished by the different arrangement of the uteri, being opisthodelphys instead of amphidelphys.

Hosts: (1) *Corvus sinensis*, (2) *Urocissa sinensis*.
Locations: (1) lung, (2) trachea.
Locality: Peiping, China.
Type specimens: Helminth. Coll., Pe. Nos. (1) 1198A, (2) 89A.

Male:	Number of specimens measured	5
	Body length	15·00–18·00
	Body width	0·14– 0·17
	Nerve ring to anterior end	0·10– 0·12
	Oesophagus: Muscular portion	0·14– 0·15
	Glandular portion	0·50– 0·51
	Spicules: Left	0·07– 0·08
	Right	0·05– 0·06
	Tail	0·20– 0·23
Female:	Number of specimens measured	5
	Body length	23·00–25·00
	Body width	0·23– 0·25
	Nerve ring to anterior end	0·11– 0·13
	Oesophagus: Muscular portion	0·16– 0·19
	Glandular portion	0·50– 0·62
	Vulva to anterior end	0·36– 0·43
	Microfilaria	0·15– 0·18
	Tail	0·18– 0·39

Extremities attenuated, especially caudad. Mouth surrounded by two pairs of sub-medial papillae and the amphids. Nerve ring at the connection between the muscular and glandular oesophageal portions, the glandular being much broader and about three times longer than the muscular. Intestine much broader than oesophagus. Posterior end of intestine of female completely atrophied and connected by a few strips of tissue to the groove of the cuticle replacing the anus. Intestine of male similarly atrophied, but cloacal opening still present as an outlet of the spicules and sperm.

Male. Tail spirally coiled, digitiform and relatively long. Caudal alae absent, three pairs of caudal papillae sometimes asymmetrically arranged. Spicules fairly stout, trough-shaped, the left being slightly longer than the right.

Female. Tail blunt and relatively long. Vulva at the anterior region of the posterior third of the oesophagus; opisthodelphys, uteri twisted around

each other in their backward course; a complete series of single cell stages to fully developed microfilariae being found from ovaries to vagina.

The present species bears a close resemblance to *Chandlerella bosei* (*Filaria bosei*) Chandler 1924. Chandler, however, had found two species of filariae, *Filaria bosei* and *Filaria brevicauda*, in the same bird, and was not absolutely sure of the correct pairing of males and females of these two species. Moreover, the description of females of both species especially in the arrangement of uteri of *C. bosei*, as given by Chandler does not agree with that of the present form. The oesophagus of the present species is definitely divided, an important character which also had not been mentioned by Chandler in his description of *C. bosei*.

Literature to foregoing section: CHANDLER, A. C. (1924). *Parasitology*, **16**, 398; (1929). *Proc. U.S. Nat. Mus.* **75**, 3.

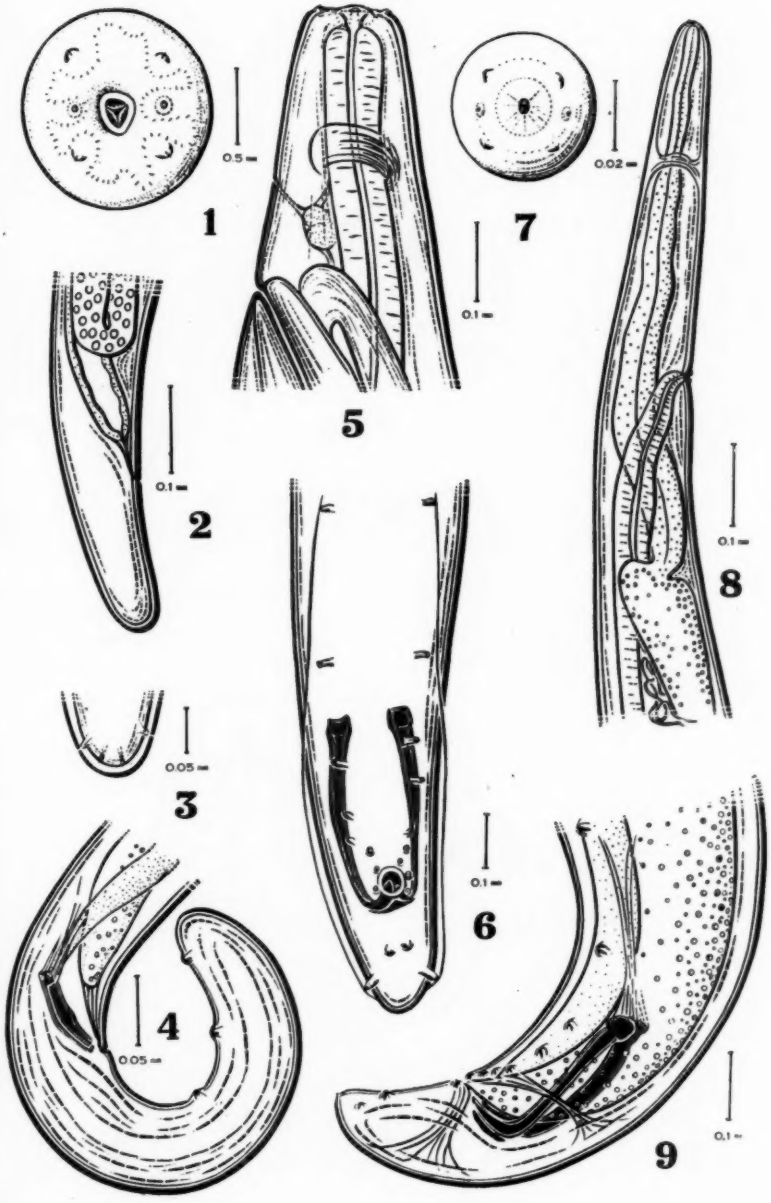
(*MS. received for publication* 17. x. 1932.—Ed.)

Legends to Figs. 1–9.

Note: All drawings were made with the aid of a camera lucida.

Figs. 1–9. *Pseudaprocta* and *Chandlerella* spp.

Fig. 1. *P. decorata* n.sp. ♀ Ant. end; frontal view.
Fig. 2. *C. sinensis* n.sp. ♀ Post. end; lateral view.
Fig. 3. *P. decorata* n.sp. ♀ Post. end; ventral view.
Fig. 4. *C. sinensis* n.sp. ♂ Post. end; lateral view.
Fig. 5. *P. decorata* n.sp. ♀ Ant. end; lateral view.
Fig. 6. *P. decorata* n.sp. ♂ Post. end; ventral view.
Fig. 7. *C. sinensis* n.sp. ♀ Ant. end; frontal view.
Fig. 8. *C. sinensis* n.sp. ♀ Ant. end; lateral view.
Fig. 9. *P. decorata* n.sp. ♂ Post. end; lateral view.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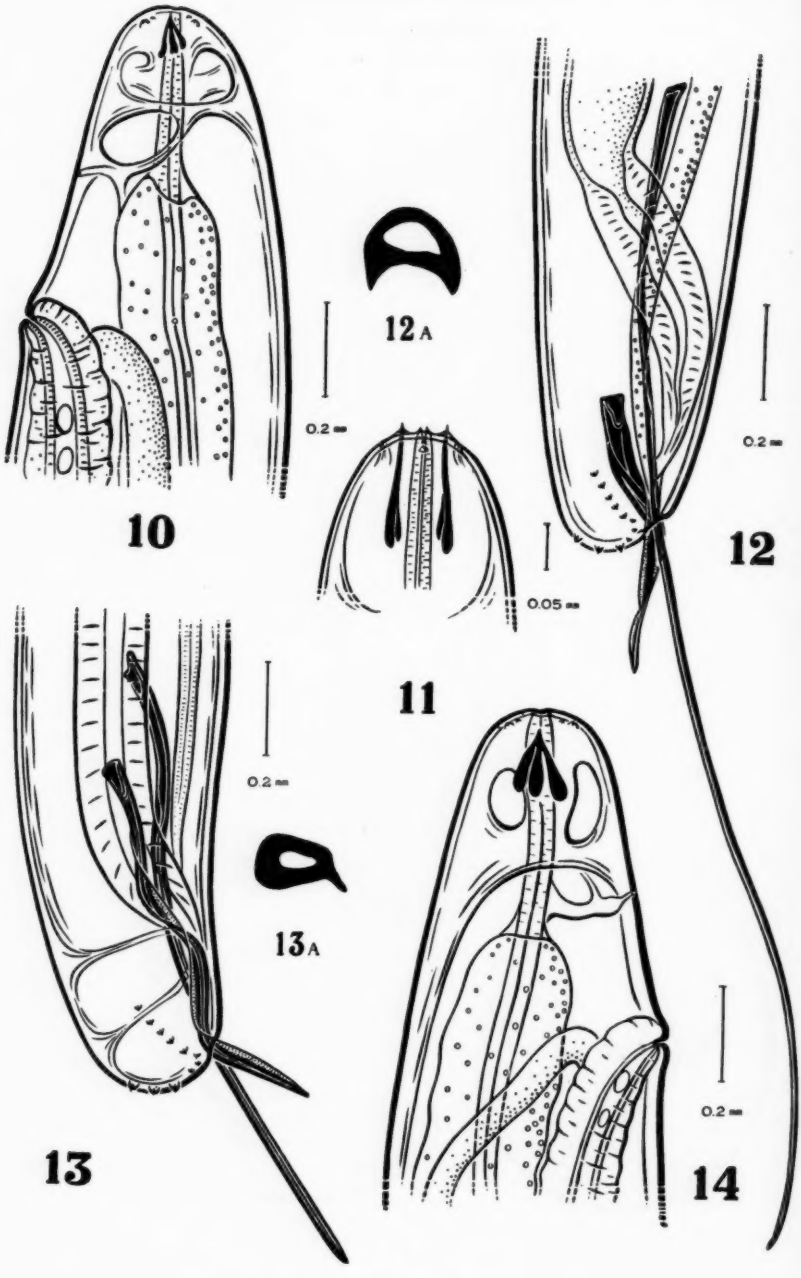
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Legends to Figs. 10–14.

Figs. 10–14. *Diplotriaena* spp.

Fig. 10. *D. tricuspis* (Fedtschenko 1847) Seurat 1915. ♀ Ant. end; lateral view.

Fig. 11. *D. diucae* Boulenger 1920. ♂ Ant. end; dorsal view: showing protrusion of tridents and lips.

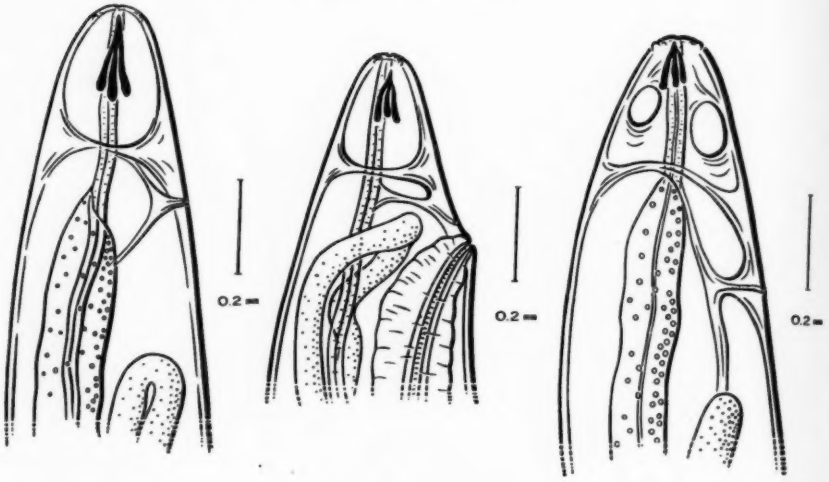
Fig. 12. *D. nochti* Hoeppli and Hsü 1929. ♂ Post. end; lateral view.

Fig. 12 A. *D. nochti*. Cross-section made at about middle of spiral spicule.

Fig. 13. *D. tricuspis* (Fedtschenko 1847) Seurat 1915. ♂ Post. end; lateral view.

Fig. 13 A. *D. tricuspis*. Cross-section made at about middle of spiral spicule.

Fig. 14. *D. nochti*. ♀ Ant. end; lateral view.



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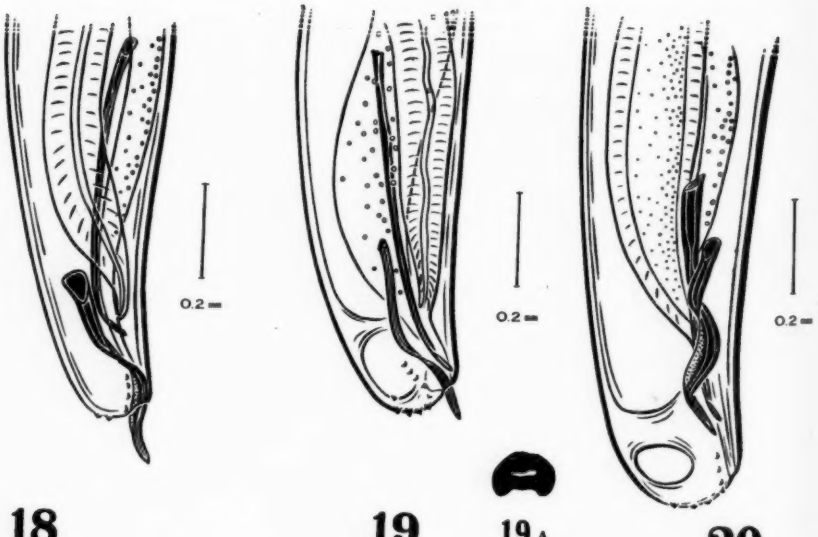
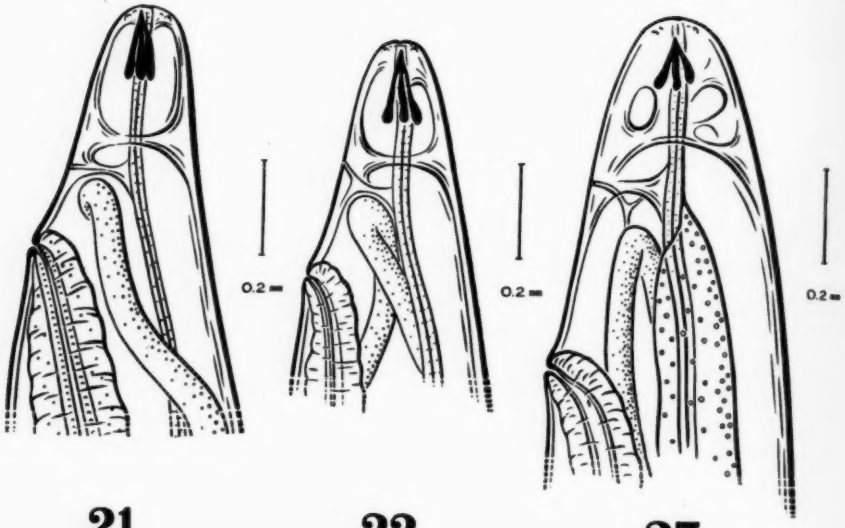


18 **19** **19A** **20**

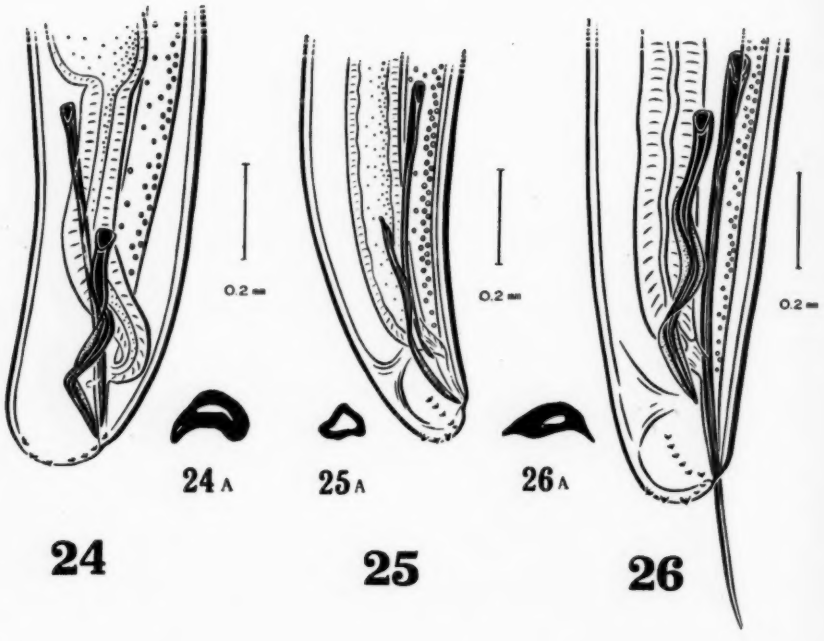
Legends to Figs. 15–20.

Figs. 15–20. *Diplotriaena* spp.

Fig. 15. *D. tridens* (Molin 1858) Boulenger 1928. ♂ Ant. end; lateral view.
Fig. 16. *D. diucae* Boulenger 1920. ♀ Ant. end; lateral view.
Fig. 17. *D. microphallos* n.sp. ♂ Ant. end; lateral view.
Fig. 18. *D. tridens.* ♂ Post. end; lateral view.
Fig. 19. *D. diucae.* ♂ Post. end; lateral view.
Fig. 19 A. *D. diucae.* Cross-section made at about middle of spiral spicule.
Fig. 20. *D. microphallos* n.sp. ♂ Post. end; lateral view.



21 **22** **23**

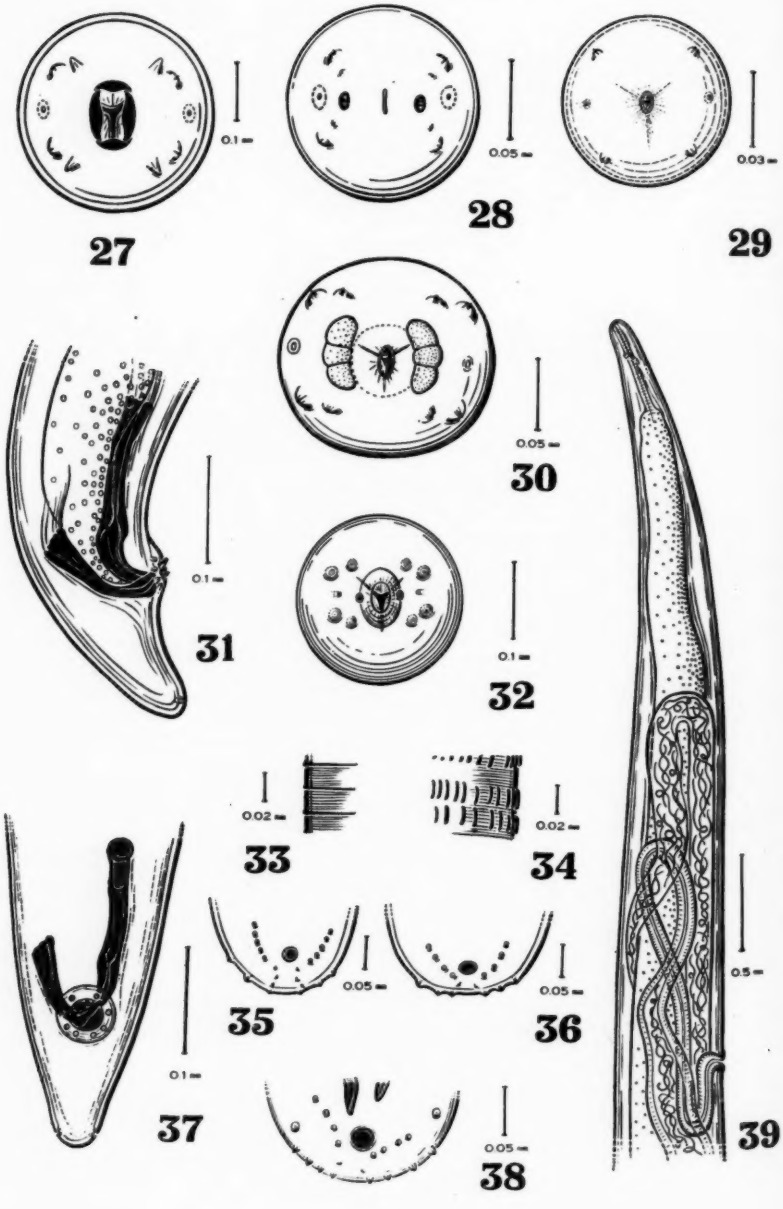


24 **25** **26**

Legends to Figs. 21–26A.

Figs. 21–26 A. *Diplotriaena* spp.

Fig. 21. *D. cantonensis* n.sp. ♀ Ant. end; lateral view.
Fig. 22. *D. alaudae* Zeder 1803. ♀ Ant. end; lateral view.
Fig. 23. *D. unguiculata* Rudolphi 1819. ♀ Ant. end; lateral view.
Fig. 24. *D. cantonensis* n.sp. ♂ Post. end; lateral view.
Fig. 24 A. *D. cantonensis* n.sp. Cross-section made at about middle of spiral spicule.
Fig. 25. *D. alaudae.* ♂ Post. end; lateral view.
Fig. 25 A. *D. alaudae.* Cross-section made at about middle of spiral spicule.
Fig. 26. *D. unguiculata.* ♂ Post. end; lateral view.
Fig. 26 A. *D. unguiculata.* Cross-section made at about middle of spiral spicule.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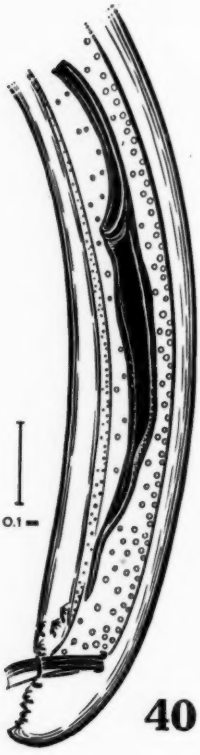
Legends to Figs. 27–39.

Figs. 27–39. *Diplotriaena, Hamatospiculum, Onchocerca, Setaria* and *Serratospiculum* spp.

Fig. 27. *Set. equina* (Abildgaard 1789) Railliet and Henry 1911. Ant. end; frontal view.
Fig. 28. *D. unguiculata* Rudolphi 1819. Ant. end; frontal view.
Fig. 29. *O. bambusicolae* n.sp. Ant. end; frontal view.
Fig. 30. *Ser. turkestanicum* Skrjabin 1916. Ant. end; frontal view.
Fig. 31. *O. bambusicolae* n.sp. ♂ Post. end; lateral view.
Fig. 32. *H. brasilianum* Skrjabin 1916. Ant. end; frontal view.
Fig. 33. *O. bambusicolae* n.sp. ♀ Annular thickenings on cuticle.
Fig. 34. *O. bambusicolae* n.sp. ♂ Rod-like cuticular thickenings on ventral surface of coiled part of tail.
Fig. 35. *D. unguiculata.* ♂ Post. end; ventral view showing caudal papillae.
Fig. 36. *D. alaudae* Zeder 1803. ♂ Post. end; ventral view showing caudal papillae.
Fig. 37. *O. bambusicolae* n.sp. ♂ Post. end; ventral view.
Fig. 38. *D. diucae* Boulenger 1920. ♂ Post. end; ventral view showing caudal papillae.
Fig. 39. *O. bambusicolae* n.sp. ♀ Ant. end; lateral view.



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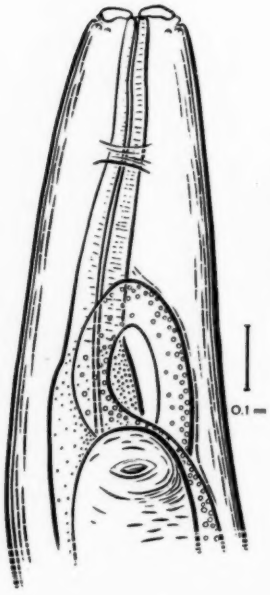

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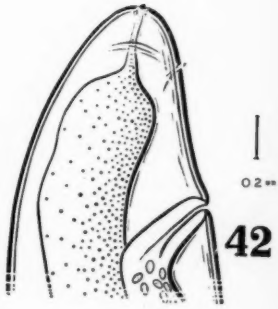

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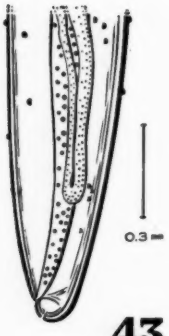

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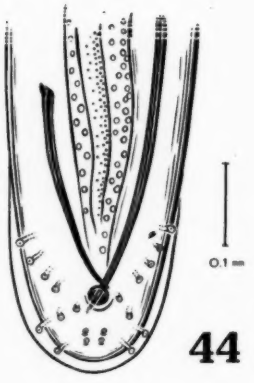

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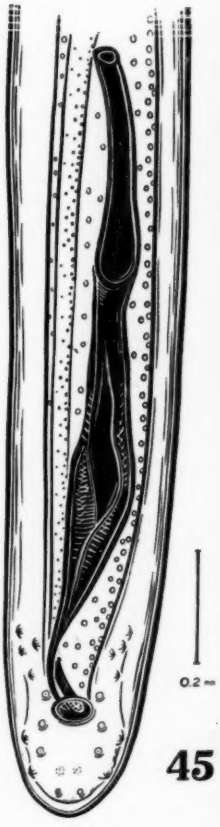

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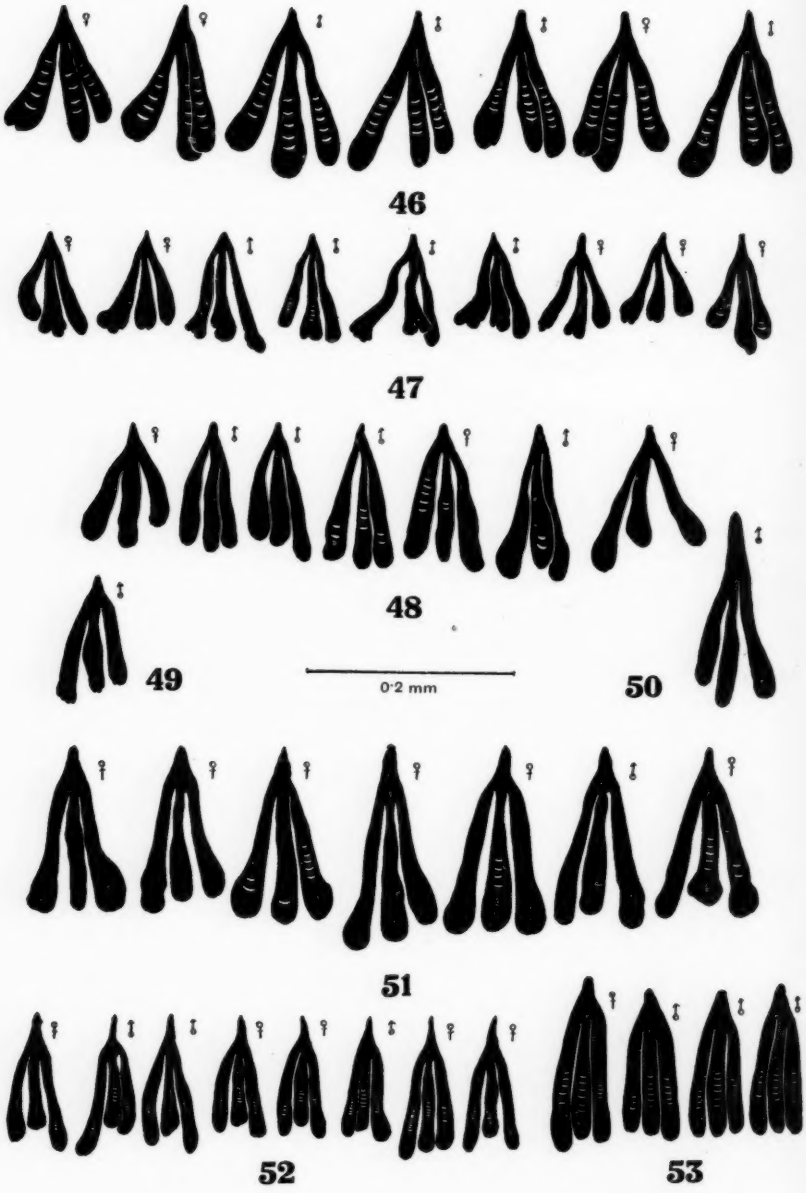
Legends to Figs. 40–45.

Figs. 40–45. *Hamatospiculum* and *Serratospiculum* spp.

Fig. 40. *S. turkestanicum* Skrjabin 1916. ♂ Post. end; lateral view (short spicule dislocated, distal end broken).
Fig. 41. *S. turkestanicum*. ♀ Ant. end; ventral view.
Fig. 42. *H. brasilianum* Skrjabin 1916. ♀ Ant. end; lateral view.
Fig. 43. *S. turkestanicum*. ♀ Post. end; lateral view.
Fig. 44. *H. brasilianum*. ♂ Post. end; ventral view (distal end of long spicule broken).
Fig. 45. *S. turkestanicum*. ♂ Post. end; ventral view (short spicule dislocated, distal end broken).


46
47
48
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0·2 mm
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Legends to Figs. 46–53.

Figs. 46–53. *Diplotriaena* spp.

Fig. 46. *D. nochti* Hoeppli and Hsü 1929. Tridents, one from each specimen.
Fig. 47. *D. tricuspis* (Fedtschenko 1847) Seurat 1915. Tridents, one from each specimen.
Fig. 48. *D. unguiculata* Rudolphi 1819. Tridents, one from each specimen.
Fig. 49. *D. microphallos* n.sp. ♂ Trident.
Fig. 50. *D. tridens* (Molin 1858) Boulenger 1928. ♂ Trident.
Fig. 51. *D. alaudae* Zeder 1803. Tridents, one from each specimen.
Fig. 52. *D. diucae* Boulenger 1920. Tridents, one from each specimen.
Fig. 53. *D. cantonensis* n.sp. Tridents, one from each specimen.

TRICHOSTRONGYLID NEMATODES FROM SHEEP IN KENYA.

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(With 17 Figures in the Text.)

In most parts of the world helminth infestations are responsible for such a heavy wastage of sheep that their control has undoubtedly become one of the most important charges that devolve upon the flock-owner. It might be thought that in tropical regions suited to the raising of sheep the problem of helminth control would cease to be urgent; and that climatic conditions, the general aridity of land chosen in such countries for sheep-rearing, and its consequently smaller carrying capacity, would contribute to form an environment distinctly unfavourable both to the survival of the free-living stages of parasitic worms and to their chances of reaching a suitable intermediary or definitive host. Such areas are not usually well watered, and it certainly does result from the paucity of permanent streams and standing water that few species of trematodes are represented. Those species that do occur, *e.g. Fasciola hepatica*, *F. gigantica*, are restricted to particular localities and are easily subjected to control.

The conditions in question do also somewhat diminish the incidence of infestations with the adult cestodes; but this group has no great economic importance and infestations with *Moniezia* and *Avitellina* appear to be largely self-limited and relatively harmless.

With nematodes the case is different, for, while certain species are undoubtedly affected adversely by a relatively arid environment, many seem able to thrive equally well under conditions approximating to either extreme; and there are still others that appear to thrive better under arid conditions than in the moister zones. It is from heavy infestation with nematodes that the most serious mortality of sheep in Kenya arises.

Climate in Kenya varies strikingly with the locality, depending upon altitude, soil and vegetation. The areas in which sheep-breeding is practised can, however, conveniently be considered as of two main types: high plateau land with a high annual rainfall well distributed throughout the greater part of the year and a preponderance of cloudy or even misty weather; and lower plains country with a relatively low annual rainfall, restricted to one or two short rainy seasons, and for the rest brilliant sunshine and a low degree of atmospheric humidity. In the former areas, situated at an altitude of from

7500 ft. upwards, the Romney Marsh breed has been fairly generally used to improve the native stock, either with or without an admixture of merino blood; and such breeds as Lincoln, Suffolk and Corriedale have been tried as sires on a smaller scale. In the plains country the stock consists almost entirely of merino grades, bred originally from native Masai or Boran ewes, and there has been practically no introduction of other blood.

From the point of view of the helminthologist the fact that importations into what we might term the Romney areas have been mainly from England and those into the merino areas from Australia and South Africa, might possibly be held responsible for differences observed between the parasitic fauna in the two areas. As against this view it may be stated that in the Romney areas merino sheep were generally tried in the early days, and even to-day merinos from the plains country are frequently moved to the Romney country when grazing in the plains is scarce as a result of prolonged drought. There has, therefore, been every opportunity for an interchange of species, but the fact remains that there are undeniable differences between the parasitic nematode fauna of sheep from the two types of country. Of the economically important species the sheep hookworm (*Bunostomum phlebotomum*), the "stomach-worm" or "wire-worm" (*Haemonchus contortus*), the nodular worm (*Oesophagostomum columbianum*) and various *Trichostrongylus* spp. are common to both groups. Two species of the genus *Ostertagia*, one an undescribed one (p. 236), are quite definitely restricted to the Romney areas. There is reason to suppose that *Ostertagia* is not an African genus and that these are introduced species. In the plains country the genus *Cooperia*, which is almost entirely unknown in the Romney areas, is largely represented, and from the common occurrence of its species in other hosts it can be considered as an indigenous group. There also appear in sheep from the plains country species of *Cooperia*, of a related genus *Cooperioides* n.g., and of the Heligmosome genus *Impalaia*, all of which are undoubtedly normally parasitic in wild ruminants. My experience during the last six years is that certain of these species from game are showing a definite tendency to establish themselves permanently in sheep. One may remark in passing that of the two types of country already described each has its characteristic fauna of wild ruminant species, the plains country having both the wider range of species and by far the greater density of individuals. Plains game is made up largely of the gregarious species of antelope and gazelle, hartebeest or kongoni, Thompson's and Peters' gazelle, impala and oryx; together with eland and wildebeest which are not so generally distributed over the sheep-breeding country. On the high and more rainy plateau country most of the remaining species are of solitary habit—bushbuck, reedbuck, duiker and waterbuck, though kongoni are found on one of the higher plateaux.

During the past seven years attention in Kenya has been mainly devoted to the more highly pathogenic species of nematodes parasitising sheep. An attempt has been made to educate flock-owners in the need for careful manage-

ment of pastures to prevent the occurrence of heavy infestations of the younger sheep, particularly weaners. The value of early diagnosis and of routine anthelminthic treatment has been repeatedly demonstrated. Considerable success has attended this campaign, and one no longer hears of the loss of 80 per cent. of the year's crop of lambs from infestations with hookworms or small trichostrongyles.

It is now possible to give consideration to less urgent matters, and one of the most interesting is this gradual increase that has been observed in the frequency with which the species, already referred to as normally parasitic in wild ruminants, are being collected from sheep. A species of *Impalaia* (*I. nudicollis*) recently described by Mönnig (1931) from a blesbuck (*Damaliscus albifrons*) captured near Pretoria, was collected from sheep in East Africa as long ago as 1926, a single male specimen being found in a collection consisting mainly of *Trichostrongylus* spp. sent by Mr Hornby from Tanganyika. In 1929 a few specimens were taken from sheep in the Rift Valley, Kenya, and in 1930 and 1931 it has not been uncommon to find upwards of a hundred specimens in sheep from this district and from the Athi Plains, south of Nairobi.

Mönnig, approaching the problem from another angle, was unable to produce infestation with this species in four lambs that were fed on two separate occasions with third-stage larvae derived from infested *Damaliscus*, and he concluded that *Impalaia nudicollis* is not transferable to lambs. Although *Damaliscus albifrons* does not occur in East Africa there is no doubt that the species of which a description is given on p. 239 is identical with Mönnig's species, and that under natural conditions it is able to infest sheep and reach maturity in that host. The description of this species (under a name which was a virtual homonym of that proposed by Mönnig) and the drawings were ready for press when Mönnig's paper came to hand. Publication of this description and of that of two other species now included in this paper had been deferred for some considerable time in the hope that their true hosts would be discovered. A second one of these species, *Cooperia serrata*, has also been described by Mönnig (1931), from a springbuck, *Antidorcas marsupialis*, captured in South Africa. A description of this species appears on p. 232. With *Cooperia serrata*, Mönnig was able to infest lambs by feeding them with the third-stage larvae reared from the faeces of two springbuck. This species has not been collected from naturally infested sheep in South Africa, but has been collected even more frequently than *Impalaia nudicollis* from sheep in the Rift Valley and Athi Plains areas in Kenya, up to fifty specimens being taken from individual sheep. *Antidorcas marsupialis* does not occur north of the Zambesi and the usual host of *Cooperia serrata* in Kenya is unknown. The third species, here considered as normally a game parasite, is *Cooperioides kenyensis*, p. 234. A new genus is proposed to accommodate this species and *Cooperia antidorca* Mönnig 1931, which is also described from the springbuck in South Africa. Mönnig was able to infest two lambs with larvae of *C. antidorca* obtained from cultures of the faeces of two recently captured springbuck. *C. antidorca* has not so far

been encountered in Kenya, but *Cooperioides kenyensis*, which is a closely related species, has been collected from sheep about as frequently as *Impalaia nudicollis* and *Cooperia serrata*, although in smaller numbers.

Mönnig, as a result of his experiments, is able to record the sheep as a new host for five species of nematodes, viz. *Haemonchus bedfordi*, *Cooperia fuelleborni*, *C. fuelleborni* var. *hungi*, *C. serrata*, *C. antidorca*, and *Setaria hornbyi*. Only the last-mentioned species is recorded as occurring naturally in sheep, all the other infestations having resulted from experimental feedings of larvae obtained from wild ruminants. Two of these species are now recorded as naturally contracted parasites of sheep in Kenya, and a form closely related to another of Mönnig's species is also described from sheep.

A survey of the helminth parasites of wild ruminants in the sheep-rearing districts of Kenya is now in progress and a report will be published at a later date.

Descriptions are also given in this paper of two other new trichostrongyles collected from sheep in Kenya, together with notes on certain other species, which may be of assistance in the determination of specimens.

TRICHOSTRONGYLIDAE Leiper 1911.

TRICHOSTRONGYLINAE Leiper 1908.

TRICHOSTRONGYLUS Looss 1905.

Trichostrongylus colubriformis (Giles 1892).

Synonyms: *Trichostrongylus instabilis* (Railliet 1893); *Strongylus subtilis* Looss 1895.

Hosts: *Ovis aries*, *Bos taurus*, *Gazella granti*.

Habitat: Duodenum and fourth stomach.

Locality: Eastern Africa generally.

This species has frequently been collected from sheep and goats in Kenya. It has been found in large numbers associated with *Trichostrongylus extenuatus* in enteritis of sheep and goats by Ransom in America and by the present writer in Great Britain. More recently it has been cited as the cause of similar symptoms in South Africa by Mönnig and others.

During the past six years several outbreaks of gastro-enteritis accompanied by severe emaciation have been investigated in Kenya sheep that have been found to be due to heavy infestation with this species and *T. probolurus*. The infestations are usually mixed ones with three or four species of *Trichostrongylus*, but *T. colubriformis* is always present in greatest number.

Although there is no previous record of the occurrence of *T. colubriformis* in cattle it has been found necessary to assign to this species many specimens from cattle in Kenya. The only respect in which the specimens collected from cattle do not agree with the accepted description of the species is in the size of the spicules and accessory piece. The spicules in the specimens from cattle, while of

the form typical for the species, vary in length from 0·111 to 0·154 mm., and a series measured will give all gradations between these two points. Examination of a large number of specimens from sheep reveals that they show an equal degree of variation in the size of these structures. The length of the accessory piece varies from 60 to 70 μ.

Trichostrongylus probolurus (Railliet 1896).

Hosts: *Ovis aries, Gazella thompsoni.*
Habitat: Duodenum.
Locality: Eastern Africa generally.

This form has been collected in varying numbers from sheep. It is commonly associated with *T. colubriformis* in heavy infestations.

Trichostrongylus axei (Cobbold 1879).

Hosts: *Bos taurus, Ovis aries, Capra hircus.*
Habitat: Fourth stomach and duodenum.
Locality: Eastern Africa generally.

T. axei is a common parasite of sheep and cattle in Kenya.

Up to the present no outbreaks of gastro-enteritis in calves have been ascribed to the presence of this parasite in Kenya, although in Great Britain losses among calves occasioned by heavy infestation with this species are by no means uncommon.

Trichostrongylus vitrinus Looss 1905.

Hosts: *Ovis aries, Capra hircus.*
Habitat: Duodenum and fourth stomach.
Locality: Eastern Africa generally.

This species is not very common in Kenya, but has been collected in small numbers from sheep and goats together with other species of *Trichostrongylus.*

Trichostrongylus hamatus n.sp.

(Figs. 1 and 2.)

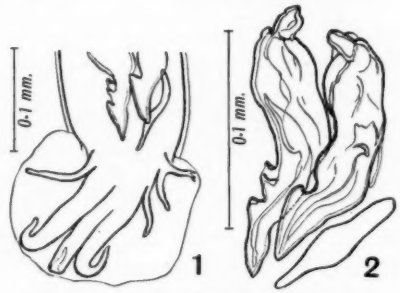
Host: *Ovis aries.*
Habitat: Fourth stomach.
Locality: Highlands of Kenya Colony.

This form has been collected frequently from sheep suffering from heavy infestations with *T. colubriformis,* and on one occasion it was found as almost a pure infestation in a sheep from one of the Romney breeding districts (Molo).

It is a small species. The male is 4–5 mm. long and up to 0·08 mm. in maximum thickness, the female is 5·5–6·5 mm. long and up to 0·08 mm. in thickness. The specific characters are found in the structure of the bursa and spicules of the male. The bursa resembles that of *T. colubriformis.* The ventro-ventral ray is very slender and widely divergent from the latero-ventral, which is approximately equal in thickness to the antero-lateral. These two are the

thickest of the rays, with the medio-lateral somewhat more slender. The postero-lateral ray is very slender, divergent from the medio-lateral, and reaches only about half-way to the edge of the bursa. The externo-dorsal arises from the main trunk of the dorsal ray at its origin, and is of about the same thickness as the postero-lateral, but does not extend as far towards the edge of the bursa. The total length of the dorsal ray including its terminal branches is 0·035–0·040 mm., and it is cleft for about one-third of its length. The terminal branches of this ray are bifid (Fig. 1).

The spicules are sub-equal, dissimilar, and curved; they are broad and foliaceous. In general appearance they resemble those of *T. probolurus*, but are readily distinguished by their inequality and dissimilarity. The left spicule is 0·125–0·135 mm. long and is furnished in its distal third with two barbs, similar in shape to the angular projections on the spicules of *T. probolurus* but not indenting the shaft of the spicule so deeply. The right spicule is shorter, 0·110–0·118 mm., and possesses only a single barb, which is more massive than



Figs. 1 and 2. *Trichostrongylus hamatus.*
Fig. 1. ♂ bursa, lateral.
Fig. 2. ♂ spicules and accessory piece.

the terminal barb of the left spicule. The accessory piece is 0·07–0·075 mm. long and is of the shape usual to the genus.

The tail of the female measures 0·06–0·07 mm. and is similar in shape to that of *T. probolurus*. The vulva is located about 2 mm. from the posterior end and has prominent lips, its total antero-postero length including the swollen lips varies from 0·022 to 0·027 mm. The combined length of the muscular portions of the ovejectors is about 0·475 mm. The eggs are segmenting when laid and measure 0·07–0·082 × 0·035–0·04 mm.

Of the members of this genus from domestic ruminants only two others have unequal spicules, *T. extenuatus* (Railliet and Henry 1898) and *T. rugatus* Mönnig 1925. The males of *T. hamatus* are readily distinguished from both these species by the structure and size of the spicules and by the fact that in both *T. extenuatus* and *T. rugatus* the postero-lateral ray of the bursa is as long as the other lateral rays and reaches to the edge of the bursa, whereas in *T. hamatus* this ray is less than half the length of the remaining lateral rays and

extends only about half-way to the free margin of the bursa. The female does not possess sufficient specific characters to enable it to be distinguished from the females of such species as *T. colubriformis* and *T. rugatus*, although the vulva is situated further forward in *T. hamatus*.

Baylis (1929) gives a key to the species of *Trichostrongylus* occurring in domesticated ruminants. The characters of the male outlined above enable *T. hamatus* to be worked easily into this key.

Genus Cooperia Ransom 1907.

Cooperia curticei (Railliet 1893).

Host: *Ovis aries.*
Habitat: Duodenum and fourth stomach.
Locality: Highlands of Kenya Colony.

This species has been collected on a few occasions from sheep.

Cooperia oncophora (Railliet 1898).

Hosts: *Bos taurus, Ovis aries.*
Habitat: Small intestine.
Locality: Highlands of Kenya Colony.

This species occurs fairly frequently in collections of worms from sheep and cattle. There is nothing to add to Ransom's (1911) description.

Cooperia punctata (v. Linstow 1907).

Hosts: *Bos taurus, Ovis aries.*
Habitat: Duodenum and fourth stomach.
Locality: Highlands of Kenya Colony.

This form has been collected from sheep and cattle in Kenya. There does not appear to be any previous record of the occurrence of this species in sheep, although it is quite a common parasite of sheep in Kenya.

Cooperia pectinata Ransom 1907.

(Fig. 3.)

Hosts: *Bos taurus, Ovis aries.*
Habitat: Fourth stomach.
Locality: Eastern Africa generally.

Specimens of *C. pectinata* have been collected frequently from cattle in Kenya. The species was described by Ransom (1907) from a few specimens collected in 1900 by Stiles in Texas. Apart from the record by the writer (1925), the only other reference to this species appears to be the statement of Travassos (1921) that he has collected occasional specimens from cattle in Brazil.

In Kenya this species is exceedingly common in cattle from the plains type of country; and although there is no previous record of *C. pectinata* from sheep it is not unusual to find in sheep from these areas a number of specimens that must be assigned to this species.

In 1925 the writer expressed the opinion that specimens from cattle might be possibly divided into two races according to the length of the spicules. Further experience fails to support this view. The spicules in specimens from cattle vary in length from 0·21 to 0·38 mm. and all gradations between these extremes can be found. The specimens collected from sheep also show variation in the length of the spicules, but the range is smaller, 0·21–0·26 mm. There is one small detail in which the spicules of this species differ from the figure and description given by Ransom (1911). This is in the presence of a slender, dorso-internal, and easily overlooked spike springing from the main shaft near the junction of its middle and distal thirds (Fig. 3). According to Ransom the length of the dorsal ray including its terminal branches, which are parallel, is at least 0·18 mm. Of the Kenya specimens those with the shortest spicules have dorsal rays measuring 0·18 mm. or more, while in the specimens with spicules over 0·35 mm. the dorsal ray and its branches may measure as much as 0·28 mm. The main stem of the ray is about equal in length to the terminal branches, which are roughly parallel and fairly close together.

Baylis (1926) assigned certain specimens collected from calves in Australia to a new species, *C. nicolli*. Bearing in mind the present writer's original view that the Kenya specimens fall into two races according to the length of the spicules, and that specimens towards the upper limit of the range would be within the spicule measurements given by Baylis for *C. nicolli*, a number of specimens was submitted to re-examination with the result already stated. Baylis' description, measurements, and figures of *C. nicolli* fit the larger specimens of *C. pectinata* perfectly, with the exception that he has not mentioned or figured the dorso-internal process found on the spicules of the Kenya specimens. Possibly an examination of a larger number of specimens from Australian cattle would show that they are subject to the same range of variation as the Kenya specimens, in which case Baylis' species would fall into synonymy.

Mönnig (1931) gives a short description of a new variety of *C. fuelleborni*, which he names *C. fuelleborni* var. *hungi*, in which the spicules measure about 0·235 mm. In general appearance the spicules resemble those of *C. pectinata*, and although Mönnig's variety may be a perfectly valid one there is nothing in the text that would serve to distinguish it from *C. pectinata*, again with the exception of the dorso-internal process of the spicule.

There is no doubt that as a species *C. pectinata* is subject to a considerable degree of variation, and that there is need for a competent revision of this and allied species by a worker with material at his disposal from Africa, Australia and America. It is possible that *C. curticei* is also a variable species and that examination of specimens of *C. curticei*, *C. fieldingi* and *C. fuelleborni* from various parts of the world would enable one to make some reduction in the number of species. At the present time there is uncertainty as to the validity of some of these species and one experiences considerable difficulty in making determinations.

Cooperia serrata Mönnig 1931.

(Figs. 4–6.)

Host in Kenya: *Ovis aries.*
Habitat: Small intestine.
Locality: Rift Valley and Athi Plains, Kenya Colony.

This species, to which a reference has already been made (p. 226), has been collected with increasing frequency during 1930, 1931 and 1932 from sheep in the areas given above. It was originally described by Mönnig (1931) from the springbuck, *Damaliscus albifrons*, in South Africa, and Mönnig was able to produce infestation in two lambs by feeding them with larvae cultivated from the faeces of springbuck. The normal host in Kenya has not yet been discovered.

The male worms are 5–6 mm. long and up to 0·12 mm. in maximum thickness; the females are 7–10 mm. long and reach about the same maximum thickness.

The cuticle has ten narrow, but distinctly pectinate lines in addition to two faint lateral lines. Transverse striation is not discernible except on the head swelling. The head is 0·033–0·035 mm. in diameter in the male and 0·035–0·045 mm. in the female. The cephalic swelling extends backwards for a distance of 0·016–0·035 mm. Small cervical papillae can be made out. The oesophagus is 0·34–0·4 mm. long.

In the male small prebursal papillae are present. The bursa is well developed but is not distinctly divided into lateral and dorsal lobes. The ventro-ventral ray is much shorter and more slender than the latero-ventral, which appears to have a common origin with the lateral rays. The antero-lateral is slightly larger than the latero-ventral and is with the externo-dorsal the stoutest of the rays. The remaining lateral rays are slender and parallel. The externo-dorsal ray is strongly arched and does not reach the margin of the bursa. The dorsal ray is long and slender and is divided in its distal fourth. The terminal branches form a roughly lyre-shaped structure and in turn they are bifurcate at their tips. Each terminal branch has a slender twig which springs from the arched portion of the branch, and is directed laterally with a tendency to bend ventrally. The spicules are heavy and well chitinised, they are bifurcate for their distal two-fifths. The dorsal process is slightly the longer and terminates in a short foot set almost at right angles to the main axis of the spicule. The ventro-internal process bears on its ventro-internal surface in its distal half seven raised tooth-like projections giving the edge the appearance of a saw. There is no accessory piece.

The tail of the female is 0·115–0·13 mm. long, tapering gradually and terminating in a slender process. The vulva is situated 1·25–1·75 mm. from the posterior end and its opening is covered by a prominent linguiform cuticular flap, which is highly refractile and almost chitinous in appearance. The combined length of the ovejectors is about 0·3 mm., and the eggs measure 0·075 × 0·04 mm.

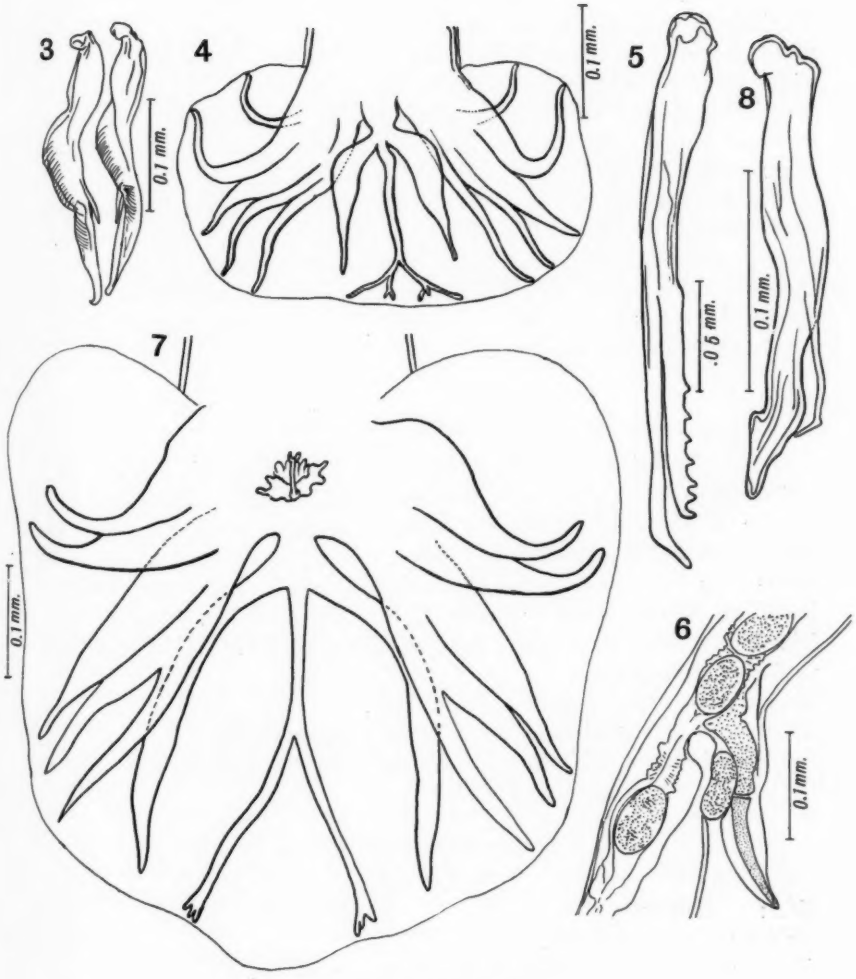


Fig. 3. *Cooperia pectinata*, ♂ spicules.

Figs. 4–6. *Cooperia serrata*.

Fig. 4. ♂ bursa, dorsal.

Fig. 5. ♂ spicule, lateral.

Fig. 6. ♀ vulvar region.

Figs. 7 and 8. *Cooperioides kenyensis*.

Fig. 7. ♂ bursa, ventral.

Fig. 8. ♂ spicule.

This species is closely related to *C. nodulosa* Schwartz 1928 from the Carabao.

In certain respects *C. serrata* is not a typical member of the genus *Cooperia*. Cervical and prebursal papillae are not present in other members of the genus, although one would expect to find them in *C. nodulosa*. The accessory branch of the dorsal ray is normally directed ventrally in *Cooperia*, and usually enters a vesicular swelling on the inside of the bursa. In addition the number of pectinate lines on the cuticle in *Cooperia* is typically sixteen, and in only one other species, *C. bisonis* Cram, is the vulva covered by a cuticular flap.

The great resemblance shown in the structure of the spicules and the dorsal ray between *C. serrata* and *C. nodulosa*, and the divergence of these two species from the accepted type of the genus would almost persuade one to place the two species in a new genus. Should a re-examination of *C. nodulosa* show that this species also is furnished with cervical and prebursal papillae, then no doubt a separate genus will be proposed.

Genus Cooperioides n.gen.

Diagnosis: Trichostrongylinae, closely related to *Cooperia*, from which it differs mainly in the structure of the bursa of the male. Ventro-ventral ray is smaller but not much shorter than latero-ventral, characteristically these two rays are not widely separated at their tips. Lateral rays as in *Cooperia*. Dorsal ray cleft for about one-half of its total length, terminal branches simple without lateral or ventral accessory branches, tips bifid or trifid. Spicules stout, foliaceous. Cuticle with twelve pectinate lines, small cervical and prebursal papillae may be present.

Habitat: Small intestine of ruminants.
Genotype: *Cooperioides kenyensis* n.sp. from *Ovis aries*.
Other species: *Cooperia antidorca* (Mönnig 1931), from the springbuck *Antidorcas marsupialis*.

Cooperioides kenyensis n.sp.

(Figs. 7 and 8.)

Host: *Ovis aries*.
Habitat: Fourth stomach.
Locality: Highlands of Kenya Colony (plains country).

This species, for which the genus *Cooperioides* is proposed, has been collected repeatedly from sheep in the Rift Valley and Athi Plains areas during the past three years. No doubt it is normally parasitic in some wild ruminant, but it may eventually succeed in establishing itself permanently in sheep. The other species assigned to the genus, *Cooperia antidorca* Mönnig 1931, was described from a captured springbuck, *Antidorcas marsupialis*, in South Africa; and Mönnig succeeded in artificially infesting a lamb to which larvae, obtained by culturing the faeces of the springbuck, had been administered.

The anterior portion of the worms may be loosely rolled spirally. The

males are 4·5–6 mm. long, and up to 0·14 mm. in maximum thickness just in front of the bursa; the females are 7·2–9·0 mm. long and up to 0·15 mm. in maximum thickness. There are twelve pectinate lines, ten of approximately equal width and two narrow and faintly marked (lateral lines). Cervical papillae appear to be absent. The head is 0·04–0·055 mm. in diameter, and the transversely striated cephalic swelling extends backwards for a distance of about 0·04–0·05 mm. The oesophagus is 0·5–0·65 mm. long.

The bursa of the male is so voluminous that males of the species can readily be picked out in the fresh state under the binocular microscope. The bursal rays have the characters of the genus, and the terminal branches of the dorsal ray are trifurcate, the outer spur obviously representing a much reduced accessory branch which is homologous with the ventral branch typical of *Cooperia*. The dorsal ray with its branches is 0·3–0·33 mm. in total length, of which the main stem of the ray is a little less than half. The spicules, which measure 0·2–0·21 mm., are stout and foliaceous. There is a dorsal and internal process springing from the main body of the spicule a little behind its middle. This slender process does not reach to the tip of the spicule, but terminates in a short hook or foot set at an angle of about 45 degrees to the long axis. The main body of the spicule also terminates in a foot-like process which has quite a characteristic form. There is no accessory piece, and prebursal papillae have not been seen. The genital cone is elaborately ornamented with cuticular thickenings.

The tail of the female is 0·15–0·19 mm. long and tapers to a fine point. The vulva is in the form of a transverse slit with swollen lips, the anterior one being especially prominent. It is situated at 1–1·9 mm. from the posterior end. The vagina is short and directed forwards at an angle of about 45 degrees. The combined length of the muscular portions of the ovejectors is 0·275–0·3 mm. The eggs measure 0·075 × 0·04 mm. The species is readily differentiated from *Cooperia antidorca* (Mönnig) by the shape of the spicules (Fig. 8), and in particular by the presence of the dorso-internal process springing from the middle of the main shaft.

The genus *Cooperia* is separated from what, apart from the new genus *Cooperioides*, must be considered its nearest neighbour, *Travassosius*, by the following characters: presence of fourteen to sixteen longitudinal pectinate lines on the cuticle; presence of a cephalic cuticular swelling; absence of cervical and prebursal papillae; division of the dorsal ray near its middle into two main branches which may form a lyre- or horseshoe-shaped structure or may run more or less parallel; presence of a latero-ventral twig which springs from each main branch, usually near its origin, to enter a vesicular swelling in the inside of the bursa.

In *Cooperia nodulosa* Schwartz and *C. serrata* Mönnig the latero-ventral twig originates low down on the main branches of the dorsal ray and the twig is hardly bent ventrally at all. In one of these species and possibly in the other also there are small cervical and prebursal papillae. The number of pectinate

lines on the cuticle is reduced to twelve, or ten if one does not include the faintly pectinate lateral lines.

In the genus *Cooperioides* the latero-ventral twig of the dorsal ray is further reduced to a terminal digitation, and the ray becomes identical in structure with that of *Travassosius*. The structure of the spicules with the foot-like termination also approaches that of *Travassosius*, but there remains the presence of the cephalic swelling and of the cuticular pectinate lines, and the absence or reduction of the cervical and prebursal papillae to distinguish the two genera.

Genus Ostertagia Ransom 1907.

Ostertagia circumcincta (Stadelmann 1894).

(Fig. 9.)

Host: *Ovis aries.*
Habitat: Fourth stomach.
Locality: Highlands of Kenya Colony.

This species, which in Great Britain is perhaps the most pathogenic of all the parasitic nematodes of sheep, has been collected on many occasions from sheep in East Africa; always in the areas where Romney Marsh rams are usually employed.

So far this species alone has not been inculpated in Kenya as the cause of severe parasitic gastritis, but it has often been present in considerable numbers in the abomasum of sheep dying from heavy infestations with *Bunostomum trigonocephalum*. Some remarks with reference to the structure of the spicules of this species are made in the course of the description of *O. pinnata* n.sp. which follows.

Ostertagia pinnata n.sp.

(Figs. 10–14.)

Host: *Ovis aries.*
Habitat: Fourth stomach.
Locality: Highlands of Kenya Colony (the so-called Romney breeding areas).

The male of this species is approximately the same size as that of *O. circumcincta*, being 7–8·5 mm. long and up to 0·11 mm. in maximum thickness. The thickest portion of the body is just anterior to the bursa.

The cuticle bears about thirty-five longitudinal ridges, spaced at roughly equal intervals. Transverse striation is extremely fine and is barely visible with an objective of 2 mm., except in the region of the head. The head is 0·02–0·024 mm. in diameter, and the papillae are not distinct. The cervical papillae are situated at about 0·37 mm. from the anterior end.

The oesophagus is about 0·7–0·74 mm. long and is encircled by the nerve-ring at about its middle.

The bursa of the male resembles very closely that of *O. circumcincta*. The ventral rays are parallel and their tips close together at the edge of the bursa. The latero-ventral ray, however, is considerably stouter than the ventro-

ventral; the former and the next two lateral rays are of approximately equal thickness, with the antero-lateral slightly the thickest of the three. The postero-lateral is divergent from the remaining lateral rays. The externo-dorsal arises from the base of the main stem of the dorsal ray and is long and

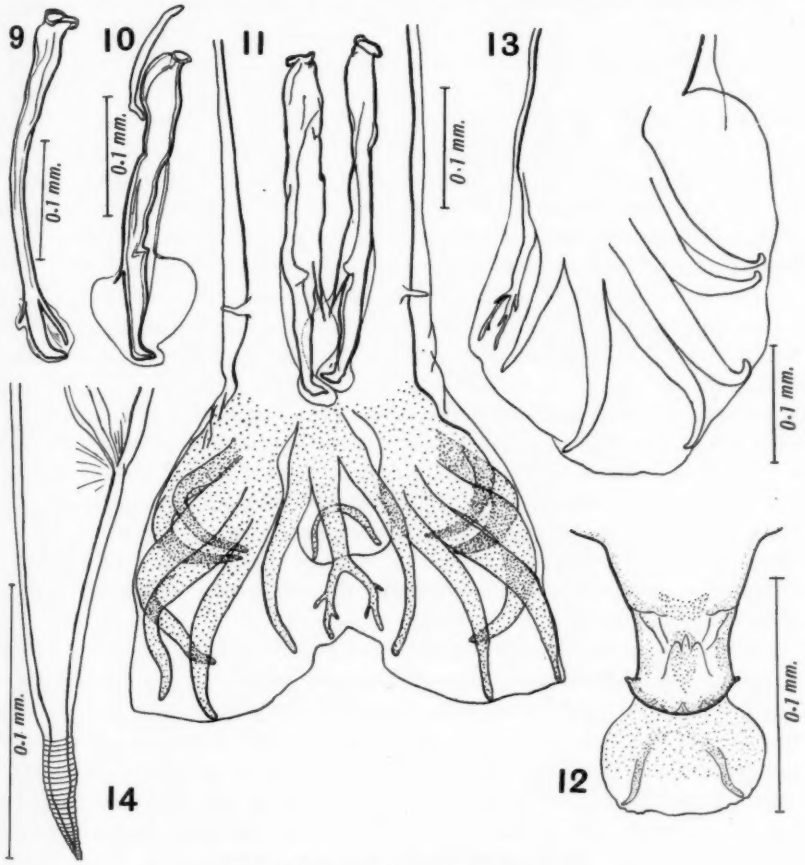


Fig. 9. *Ostertagia circumcincta*, ♂ spicule.

Figs. 10–14. *Ostertagia pinnata*.

Fig. 10. ♂ spicule.
Fig. 11. ♂ bursa, dorsal.
Fig. 12. ♂ genital cone and accessory lobe of bursa with supporting rays.
Fig. 13. ♂ bursa, lateral.
Fig. 14. ♀ tail.

relatively stout. The main stem of the dorsal ray is longer and stouter than that of *O. circumcincta*. Its length is about 0·1 mm., and that of its branches 0·05 mm. Each main branch bears a short external branch and an internal spur close to its tip. The accessory internal lobe of the bursa is not very well

developed, but its supporting rays can be recognised. Prebursal papillae are present.

The spicules afford the readiest means of differentiating the males of this species from those of *O. circumcincta* in that they are shorter, thicker, and more foliaceous in appearance than those of the latter species, and the main shaft is not curved. They are 0·22–0·265 mm. long, as against 0·28–0·32 mm. for *O. circumcincta*, and have a twisted appearance due to the development of flanges. At about the junction of their middle and distal thirds there is a dorsal and ventral process. The dorsal process is short, blunt, and directed dorsally and internally almost at a right angle to the main axis of the spicule. The ventral process is longer and more pointed: it arises a little nearer to the tip of the spicule (Fig. 11). When the spicules are exserted it is seen that there is a large membranous expansion springing from the main shaft at the level of origin of the processes and extending beyond the tip (Fig. 10).

The structure of the spicules is essentially similar in plan to that of *O. ostertagi*, *O. marshalli*, *O. trifurcata*, *O. tricuspis*, and *O. turkestanica*, in all of which the shaft is described as being trifurcate distally. According to Ransom (1911) the spicules of *O. circumcincta* are bifurcate distally, but Fig. 9 will show that when extruded the spicules of *O. circumcincta* prove to be trifurcate distally and that they resemble in general plan those of *O. pinnata*. They bear a small dorsal process and a longer and more slender ventral process, which support a membranous expansion. When the spicules of *O. circumcincta* are not exserted only the ventral and internal process can be seen. It appears probable that typically the spicules in the genus *Ostertagia* are trifurcate distally and *O. circumcincta* forms no exception to this rule. The accessory piece is lozenge-shaped and pale in colour: it is 0·09–0·1 mm. long.

On each occasion that male specimens of *O. pinnata* have been collected numerous specimens of *O. circumcincta* have also been present in the material, and it has not been possible in these mixed collections to discover any female worms that could definitely be assigned to the former species. A certain number of females was observed in which the annular striations on the tail were not confined to the slightly swollen portion of the tail but extended right to its tip. There were more than twenty striations discernible in these specimens as against the three to five generally present in the females of *O. circumcincta* (Fig. 14). In all other respects these female specimens were indistinguishable from normal females of *O. circumcincta*, and the writer does not, on this meagre evidence, feel justified in assuming that they are the females of *O. pinnata*.

The males of this species then can be distinguished from those of other species of *Ostertagia* occurring in domesticated ruminants by the length and form of the spicules and by the characters of the dorsal ray.

During the last few years several new species have been added to this genus. Some of them unfortunately have been described in relatively inaccessible publications, and difficulty may be experienced in assigning specimens when abstracts of descriptions only are available for consultation.

Variations in the thickness and form of the root, shaft and terminal processes of the spicules are difficult to indicate concisely in a table or key or even in an abbreviated text, and it may be necessary to consult the original or other figures in order to be quite certain of the determination.

The following is a list of the twenty-nine species already assigned to the genus OSTERTAGIA:

1 *ostertagi* (Stiles 1892).
2 *circumcincta* (Stadelmann 1894).
3 *pinnata* n.sp.
4 *brigantiaca* Blanchard 1909.
5 *occidentalis* Ransom 1907.
6 *trifurcata* Ransom 1907.
7 *tricuspis* Marotel 1910.
8 *marshalli* Ransom 1907.
9 *lyrata* Sjöberg 1926.
10 *mentulata* Railliet and Henry 1909.
11 *turkestanica* Petrov and Schachovzeva 1926.
12 *bullosa* Ransom and Hall 1912.
13 *orloffi* Sankin 1930.
14 *gruehneri* Skrjabin 1930.
15 *houdemieri* Schwartz 1926.
16 *skrabini* Kamensky 1929.
17 *callis* Travassos 1914.
18 *arctica* Mitzkewitsch 1929.
19 *ransomi* Travassos 1921.
20 *delicata* Travassos 1921.
21 *khalili* Travassos 1928.
22 *appendiculata* Travassos 1928.
23 *asymmetrica* Ware 1925.
24 *schulzi* Rajewskaja 1930.
25 *bisonis* Chapin 1925.
26 *harrisi* Le Roux 1930.
27 *odocoilei* Dikmans 1931.
28 *cervi* Cameron 1931.
29 *bohmi* Gebauer 1932.

HELIGMOSOMINAE Travassos 1914.

Genus IMPALAIA Mönnig 1923.

Impalaia nudicollis Mönnig 1931.

(Figs. 15–17.)

Host: *Ovis aries.*
Habitat: Fourth stomach and small intestine.
Locality: Mpapwa, Tanganyika; Rift Valley and Athi Plains, Kenya Colony.

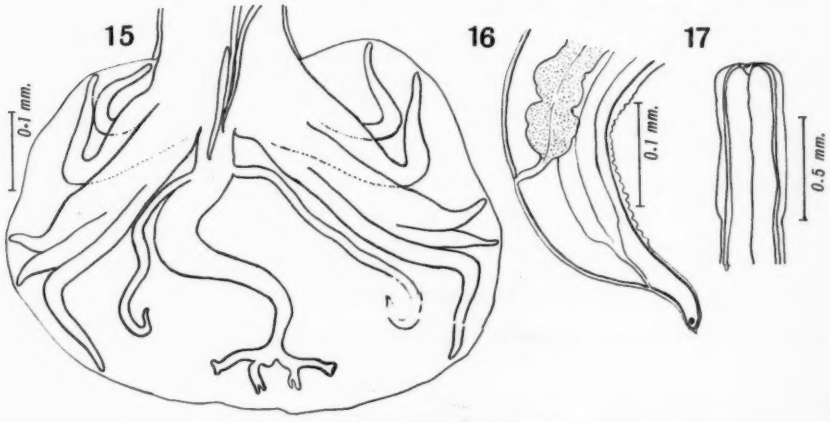
A single male specimen belonging to the genus *Impalaia* was present in material forwarded by Mr Hornby of the Mpapwa Laboratory in 1926 from the small intestine of a sheep suffering from an extremely heavy infestation with *Trichostrongylus colubriformis*. Although a careful look-out was kept for the males, which are readily recognisable under the binocular, the species was not seen again until 1929, when a few specimens were obtained from sheep in the Rift Valley. During 1930 and 1931 this species has become quite a common parasite of sheep of all ages in the Rift Valley and Athi Plains areas. There is no doubt that *Impalaia nudicollis* is normally a game parasite, and up to date it has not been collected from its normal host in East Africa. Mönnig originally described the species from *Damaliscus albifrons*, the blesbuck, in South Africa, and was unable to infest sheep by feeding larvae raised from cultures.

It may be remarked that during the seven years spent by the writer in Kenya examinations have been made of the helminth fauna of sheep from these areas at fairly frequent intervals, and that it is quite certain that during the

last two or three years this species has made considerable progress in adapting itself to the sheep as a normal host.

The male worms are 7·5–9 mm. long and up to 0·1 mm. in maximum thickness just anterior to the bursa, the females are 11–13 mm. long and up to 0·2 mm. thick. The worms are not coiled spirally, and the cuticle shows fine longitudinal markings. The head is about 0·03 mm. in diameter in the male and up to 0·045 mm. in the female. The cephalic cuticle is slightly inflated for a distance of about 0·1–0·13 mm., but there are no tubercles or small papillae in the cervical region such as are present in the other species of the genus, *Impalaia tuberculata*. The oesophagus is 0·35–0·45 mm. long.

The bursa of the male is long and voluminous, with developed lateral lobes. The dorsal lobe is not separated from the lateral lobes although the main stem



Figs. 15–17. *Impalaia nudicollis.*

Fig. 15. ♂ bursa, dorsal.
Fig. 16. ♀ posterior end, lateral.
Fig. 17. ♀ head.

of the dorsal ray is long and comparatively stout. The general arrangement of the bursal rays agrees with that of the genotype. The ventro-ventral ray is smaller than and divergent from the latero-ventral. The antero-lateral and medio-lateral rays appear to arise from a common trunk and are shorter than and distally divergent from the postero-lateral ray. The externo-dorsals are long and slender and originate from the main dorsal stem at slightly different levels, the right one higher than the left. The right externo-dorsal is roughly one-third longer than the left. The main stem of the dorsal ray is very long and fairly stout; it is characteristically curved. Measured in a straight line its total length varies from 0·4 to 0·5 mm. Mönnig does not state the measurement of the dorsal ray in his description of *I. tuberculata*, nor is any scale attached to his figures. Yorke and Maplestone (1926) give an original figure of the bursa of *I. tuberculata* in which according to the magnification table given the dorsal ray

appears to be about 0·23 mm. measured in similar fashion. The main branches of the ray are short, and their terminations bifurcate. The outer terminal branches are recurved and are longer than the inner ones, each of which gives off a short internal spur at about its middle.

The spicules are 0·9–1·0 mm. long, and are slender and filiform. There are no terminal hooks as figured by Mönnig for *I. tuberculata*. The accessory piece is boat-shaped, pale brown, about 0·085 mm. long.

The tail of the female (Fig. 16) is short and bluntly pointed or truncate at the tip, 0·055–0·06 mm. long. There is a terminal papilla and a pair of lateral sub-terminal papillae directed somewhat ventrally. The vulva is situated about 0·2 mm. from the posterior end, and the combined length of the anteriorly directed vagina and ovejector varies from 0·5 to 0·7 mm. according to the number of eggs in the uterus, and the consequent compression of the ovejector. The eggs are about 0·078 × 0·04 mm., and are segmenting when laid. They are crowded in the grand uterus without any arrangement in rows. The single ovary is confined to the anterior third of the body length.

The absence of tubercles in the cervical region, and the difference in length of dorsal ray and spicules, are characters that enable *I. nudicollis* at once to be distinguished from its congener *I. tuberculata*.

0.5 mm.

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THE LYMPH SYSTEM OF *ZYGOCOTYLE LUNATUM* (TREMATODA, PAR AMPHISTOMIDAE).

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(With 3 Figures in the Text.)

The importance of the lymph system of amphistomes and certain monostomes for purposes of classification has been emphasised by some workers, while others have considered it too intricate for practical purposes. Looss (1912) considered the lymph system to be one of the most important and characteristic structural features of the amphistomes, and he announced that for several years he had been working on a system of classification of amphistomes based in part on these structures. At that time, the structure of the lymph system was known only from the work of Looss (1902, 1912) on a few species from reptiles and mammals. More recently, contributions of Stunkard (1917, 1929) and Bhalerao (1931) have included descriptions of the lymph system in other amphistomes from reptiles and mammals, and Willey (1930) has described the system in forms from fish and amphibian hosts. *Zygocotyle lunatum* occurs in birds, and the present paper constitutes the first description of a lymph system in an amphistome from that class of vertebrate hosts.

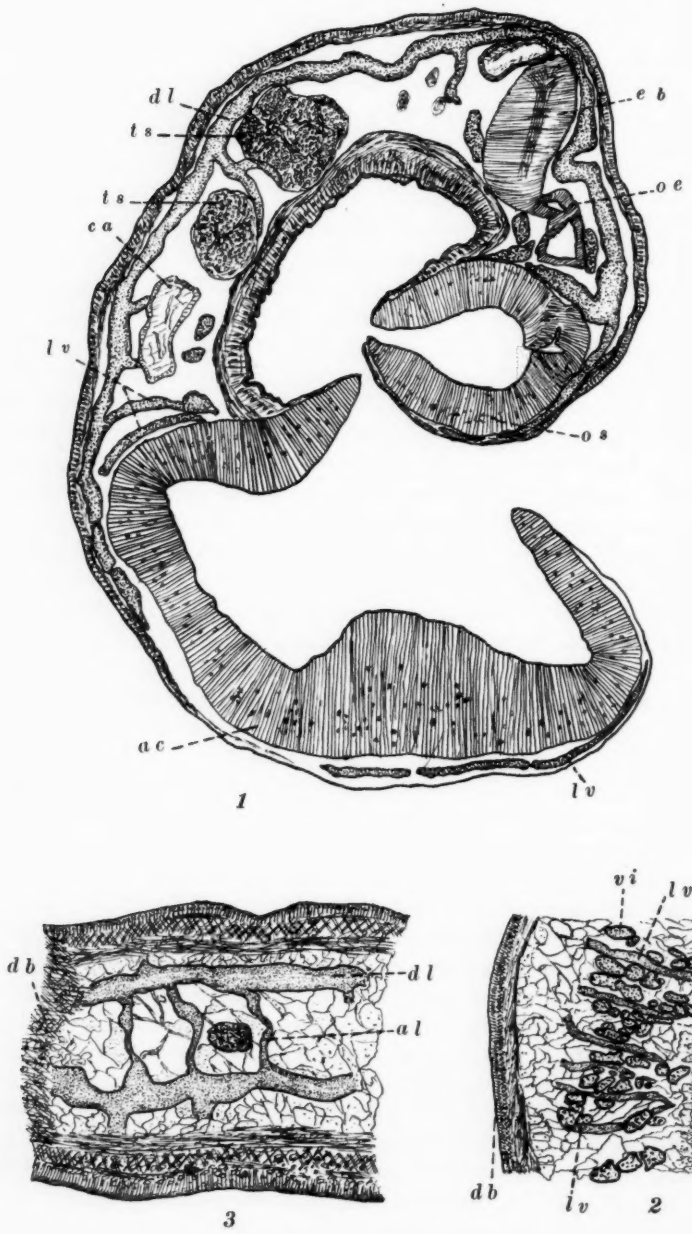
Part of the material for the present study was obtained through the courtesy of Dr M. C. Hall, Chief of the Zoological Division, Bureau of Animal Industry, and consisted of two specimens obtained from *Anas boschas* in Montana, two worms from the domestic goose, *Anser anser domesticus*, at Washington, D.C., and three specimens from the caecum of a cow, *Bos taurus*, in Panama. Thanks are also due to Prof. H. W. Stunkard for the loan of two specimens of *Zygocotyle lunatum* from the intestine of a duck, *Anas platyrhynchus*, from Nebraska. The material consisted of serial sections of eight worms, six sagittal series and one each of frontal and transverse series. Sagittal sections afforded the most favourable material, because the worms are strongly curved ventrally. Formalin preservation was followed by overnight fixation in Helly's fluid and the sections were stained in haematoxylin and eosin.

Morphology of the lymph system.

The lymph system in *Zygocotyle lunatum* consists of a single pair of main longitudinal vessels which extend the length of the body, dorsal and slightly median to the intestinal caeca, with branches running from these main channels to all parts of the worm. The main channels run forward parallel to each other to the level of the bifurcation of the gut, where they bear slightly

mediad to come very close together and extend to the anterior end of the body. At the level of the anterior margin of the acetabulum, the main vessels branch out fan-wise to surround the acetabulum with an almost continuous series of broad anastomosing sinuses, in which the tissues of the sucker are bathed. At the anterior end of the body the main channels break up in much the same manner around the oral sucker, except that the lateral sinuses are very large and almost contiguous. Between the two extremities of the body the main vessels give off numerous ventral branches. A definite branch from each main vessel can be traced to the ventral side of the body just anterior to the acetabulum, where it breaks up into branches which appear to be closely associated with the excretory structures in this region. Other definite vessels, uniformly present in all specimens, can be traced to the ovary, to the testes and to the regions of the genital openings and oral evaginations. Fig. 1 shows as much of these vessels as appeared in a series of eight sagittal sections of 10 microns in thickness, from which the reconstruction (Fig. 1) was made. In addition to these vessels which are regularly present, there are a number of branches which appear to vary in number in different specimens. These extend ventrally on either side of the intestinal caeca, give rise to sinuses which closely invest the walls of the caeca and pass to the ventral body wall in which they ramify. They may be seen passing among the lobes of the vitellaria (Fig. 2) as very definite channels which break up rather abruptly in the ventral part of the body. These vessels vary in number from ten to fourteen from each main vessel, depending on the size of the worm. The vessels in the body show frequent anastomoses, so that in places the system appears as a complex network. The main longitudinal channels show frequent transverse communicating vessels which permit a direct flow of lymph from one side of the worm to the other (Fig. 3). In addition to the definite lymph vessels, certain cavities in the body seem to contain lymph spaces which cannot be linked up with any of the main vessels or branches and hence appear as independent intercellular lymph spaces. These isolated sinuses may represent a stage in the formation of definite lymph vessels by the coalescence of such adjacent spaces as described for other forms (Willey, 1930). The size of the vessels appears to vary considerably in different specimens and sometimes in different sides of the same worm. This is probably due to contraction of the tissues around the vessels reducing the size and sometimes completely obliterating the lumen, making it difficult to trace all the vessels in a single specimen.

Histologically the lymph vessels of *Zygocotyle* are similar to those described by the writer (1930) for amphistomes of fish, Amphibia and mammals. The walls appear to be entirely membranous in structure, possessing no muscle fibres, nuclei or cell boundaries of any sort. Movement of the fluid in the channels is brought about by the contraction of the body wall and of the parenchyma. Distributed through the characteristic granular coagulum of lymph are numerous scattered corpuscles which stain strongly with basic dyes. These cells regularly contain two or three deeply staining structures



Figs. 1–3. *Zygocotyle lunatum* (camera lucida drawings).

around which networks of discrete, strongly basophilic granules appear. The corpuscles are spherical or very slightly ovoid in shape and measure an average of 5 microns in their largest diameter. They are much smaller than those of other forms in which the lymph system has been described, and are of approximately uniform size in all specimens, large or small, of the same species. The lymph corpuscles of *Diplodiscus temporatus* and of *Paramphistomum stunkardi* measure 12 microns, and those of *Cotylophoron cotylophorum* are 9·6 microns in diameter. The constancy of structure and specific form and size of these corpuscles in the lymph systems of amphistomes strongly suggest cellular elements of vascular systems; on further examination these cells may prove to be related to the lymphocytes of higher animals.

DISCUSSION.

Stunkard (1917) transferred *Amphistoma lunatum* to the genus *Zygocotyle* and suggested that a new sub-family should be created to contain it; he refrained from doing so, however, partly due to the lack of information concerning the character of the lymph system. Ward (1917) erected the new sub-family Zygocotylinae, and Fukui (1929) defined it as containing the single genus *Zygocotyle*. Bhalerao (1931) described the new genus and species *Stunkardia dilymphosa* and placed it with *Zygocotyle* in the sub-family Zygocotylinae. On the basis of the structure of *Stunkardia dilymphosa*, Bhalerao found it necessary to modify the definition of the sub-family. He described two pairs of lymph vessels in the new species, as indicated by the specific name, and therefore, in the new diagnosis of the sub-family he includes: "Lymphatic system with a pair of dorsal and a pair of ventral lymph canals." However, the lymph system had not been described for *Zygocotyle*, the type genus. He says, "Excretory and lymphatic systems are not known in *Zygocotyle*, but it is probable that they resemble those of *Stunkardia mihi*." The present research has shown that *Zygocotyle* has only a single pair of longitudinal lymph channels, as might reasonably have been expected from a knowledge of the host relationships of the genus, which is found in both birds and mammals. The lymph system in all mammalian amphistomes in which it has been investigated has shown only a single pair of main channels (Looss, 1912;

Explanation of figs. 1–3.

Fig. 1. Reconstruction of a series of eight sagittal sections (10 μ) a little to one side of the median plane.

Fig. 2. Sagittal section through vitellaria in middle third of body, showing dorso-ventral lymph vessels.

Fig. 3. Portion of a frontal section slightly ventral to the dorsal body wall, showing longitudinal lymph canals with anastomosing vessels.

Lettering: *ac*, acetabulum; *al*, anastomosing vessel; *ca*, caecum; *db*, dorsal body wall; *dl*, dorsal lymph vessel; *eb*, oesophageal bulb; *lv*, lymph vessel; *oe*, oesophagus; *os*, oral sucker; *ts*, testis; *vi*, vitellaria.

Stunkard, 1929; Willey, 1930). The occurrence of *Zygocotyle* in both birds and mammals was definitely shown by Price (1928), confirming the observations of Diesing (1836) who described the amphistome, *Amphistoma lunatum*, from the caecum of a ruminant, *Cervus dichotomus*, and also from the caeca of the birds, *Anas melanotus*, *A. ipecutiri* and *Himantopus wilsonii*. Fischoeder (1903) after an examination of Diesing's specimens concluded that the record of *Cervus dichotomus* as a host of this form was probably due to an error in labelling. However, the observations of Price (1928), showing *Bos taurus* as a host of *Zygocotyle*, have validated Diesing's original record. That part of Bhalerao's diagnosis concerning the host relationships of the sub-family Zygocotylinae, "Parasites of birds and reptiles," must therefore be changed to include parasites of mammals. In addition, the citation with respect to the lymph system in the sub-family must be modified or else eliminated entirely from the definition.

With the exception of *Stunkardia dilymphosa* Bhalerao 1931, it has been possible to show a definite correlation between the type of host and the number of main lymph channels in amphistomes in which the system has been investigated. The amphistomes of the cold-blooded hosts, fish, Amphibia and reptiles, exhibit three pairs of channels and those from warm-blooded hosts, birds and mammals, show only one pair of such channels. Among the amphistomes of reptiles, Looss (1902) described three pairs of lymph channels for *Amphistomum spinulosum* from a turtle and the same number in 1912 for *Schizamphistomum scleroporum* from turtles. Stunkard (1917) found three pairs of lymph vessels in *Allassostoma magnum* and *Allassostoma parvum* from turtles. Bhalerao's findings (1931) of only two pairs of vessels in *Stunkardia dilymphosa* would seem to point to the conclusion that the lymph system cannot be used as a basis for classification until it has been worked out in more genera. The possible importance of the system, however, for classification still exists, since the patterns of the longitudinal channels, as to number and distribution, form definite groups which can be correlated with other structural differences in the main subdivisions of the Paramphistomidae.

Summary.

The lymph system of *Zygocotyle lunatum* consists of a pair of longitudinal vessels dorsal and slightly medial to the intestinal caeca with branches to various body organs. On the basis of the lymph system and host relationships of the type genus *Zygocotyle*, the diagnosis of the sub-family Zygocotylinae as modified by Bhalerao to contain *Stunkardia dilymphosa* must be revised.

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ON *CERATOTREMA FURCOLABIATA* N.G. ET N.SP. AND *HEMIPERA SHARPEI* N.SP., TWO NEW DIGENETIC TREMATODES OF BRITISH MARINE FISHES.

By E. IDRIS JONES, B.Sc. (Lond.).

(With 9 Figures in the Text.)

Ceratotrema furcolabiata n.g. et n.sp.

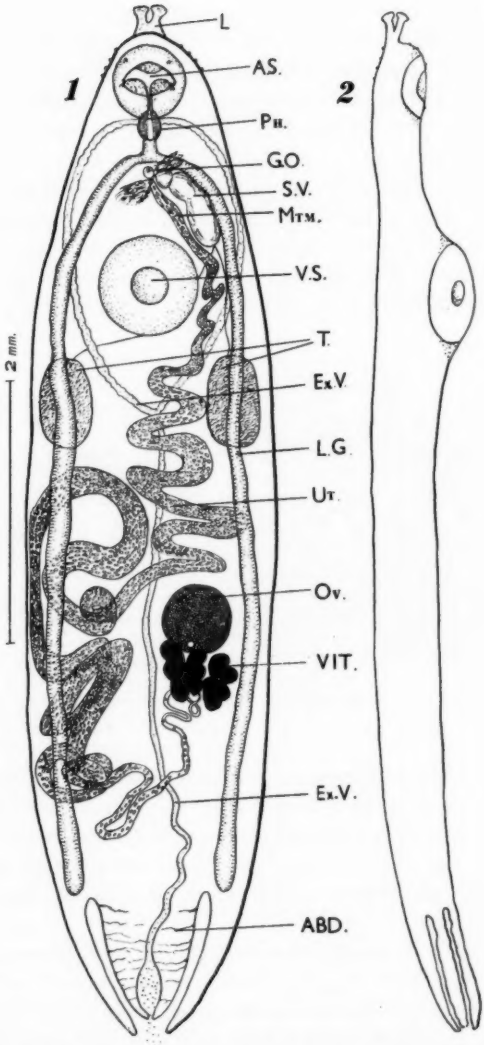
Material and habitat. Various Entozoa have been recorded from time to time occurring in the body cavity of their hosts, but very few records have been made of trematodes living in the coelom of fishes. Looss (1902) gives an account of *Probolitrema richiardi* (Lopez) occurring in the coelom of *Mustelus vulgaris* and *Acanthias vulgaris*, and this is the only form occurring in British marine fishes to which reference can be found in the literature (Nicoll, 1915).

On 1. ix. 1932 four specimens of *Onos mustela* obtained from rock-pools in Wembury Bay, Plymouth, were examined for trematodes. All contained *Hemipera ovocaudata* Nicoll 1913 in the mouth, and one contained fourteen specimens of a large trematode, hitherto undescribed, in the coelom. The parasites were large and easily discernible, reddish brown in colour, mottled with green. They were attached to the parietal peritoneum, the liver, the intestine, and along the course of the portal vein. Cestode cysts were present under the peritoneum but no cestodes, nematodes or trematodes were found in the alimentary canal. The fish was 14 cm. in length and appeared healthy.

It is proposed to make this trematode the type of a new genus and species under the name *Ceratotrema furcolabiata*.

The specimens obtained were large and had an average length of 7·7 mm. and greatest width of 1·7 mm. One of the most striking features of the species, and one which readily distinguishes it from the closely allied genus *Lecithochirium*, is the form of the lip. This lip arises by a broad base from the dorsal surface at the anterior end of the animal and terminates in two elongated processes which are very muscular and capable of great expansion and contraction. This structure is the homologue of the oral lip of the Sterrhurinae, but is more muscular and better developed than in other members of the sub-family, and does not overhang the mouth even when contracted. Its shape is seen in Figs. 1 and 2.

The general form of the animal is elongate, and bluntly rounded at both ends. At the posterior end is the short abdomen which is always invaginated into the body. The anterior sucker is sub-terminal and has three cushion-like projections into its cavity. One on the anterior wall is poorly developed, but



Figs. 1, 2. *Ceratotrema furcolabiata.* Ventral and lateral views.

ABD. abdomen; *A.S.* anterior sucker; *Ex.V.* excretory vesicle; *G.O.* genital opening; *L.* lip; *L.G.* limb of gut; *MTM.* metraterm; *OV.* ovary; *PH.* pharynx; *S.V.* vesicula seminalis; *T.* testis; *UT.* uterus; *VIT.* vitellaria; *V.S.* ventral sucker.

two postero-lateral ones are prominent and rounded. The average diameter of the anterior sucker is 0·57 mm. The ventral sucker is large and situated in the anterior third of the body and has an average diameter of 0·77 mm. Immediately anterior to the ventral sucker is the ventral groove described by Looss (1907) in the Sterrhurinae. In this species it is not transversely situated but is in the form of a pit. It has a layer of cells with deeply staining nuclei at its base. It is not obvious in the living animal or in whole mounts, but is well seen in section (Fig. 3).

The pharynx is elongated and measures 0·24 × 0·16 mm. There is no prepharynx and the oesophagus is very short, leading directly into the two limbs of the gut which in the living animal appear as a pair of longitudinal reddish brown streaks, passing down ventral to the testes and the coils of the uterus, and terminating blindly at the level of the invaginated abdomen.

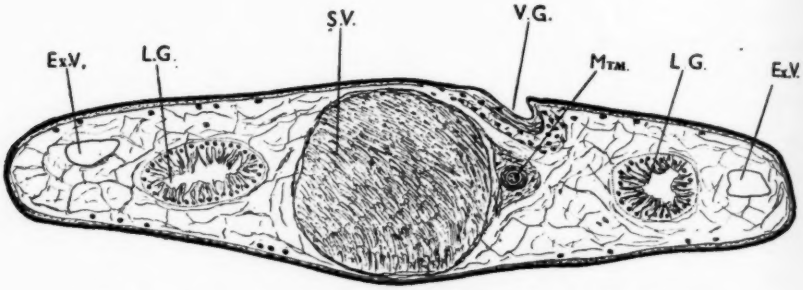


Fig. 3. *Ceratotrema furcolabiata.* Transverse section through ventral groove.

Ex.V. excretory vesicle; *L.G.* limb of gut; *MTM.* metraterm; *S.V.* vesicula seminalis; *V.G.* ventral groove.

The excretory system is typical of the Hemiuridae. It opens at the apex of the abdomen, there being a dilatation of the vesicle immediately before it opens to the exterior. The common stem of the vesicle branches at the level of the testes and each limb passes round the ventral sucker and unites with its fellow dorsal to the pharynx. The excretory granules are small and non-refractile.

The testes are two in number measuring 720 × 450 μ and are oval in shape, situated opposite each other just posterior to the ventral sucker. From the anterior pole of each a fine vas deferens passes forward to enter the vesicula seminalis, which is a dilated sac reaching to the level of the anterior border of the ventral sucker. The pars prostatica is short.

The ovary is spherical, situated near the mid-line some distance posterior to the testes. It has a diameter of 510 μ and from its posterior border emerges a narrow oviduct.

The receptaculum seminis is small and lies posterior to the ovary. From its left border emerges a duct which joins the oviduct, which then proceeds

dorsal to the vitellaria and widens to form the oötype (Fig. 4). From the oötype the uterus emerges as a narrow tube deeply staining with haematoxylin and containing few eggs in this portion. As it passes posteriorly it widens and becomes packed with eggs. In the living animal the uterus is seen as a much-coiled tube containing thousands of minute oval green eggs $23 \times 11\mu$ and occupying the greater portion of the middle of the body. Coils of the uterus are found in the posterior region of the body reaching to the level of the abdomen but none enter it. Anteriorly the uterus leads into the narrow metraterm at the level of the ventral sucker. The metraterm passes ventral to the vesicula seminalis to open with it into the genital sinus. The homology of this genital sinus or "ductus hermaphroditus" has been discussed by Manter (1926), and it is proposed to adopt his term for the muscular sac surrounding the genital sinus and call it the *sinus sac*. The genital opening is

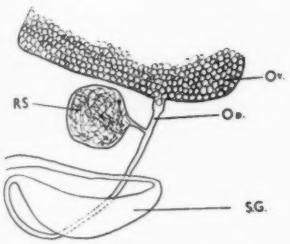


Fig. 4.

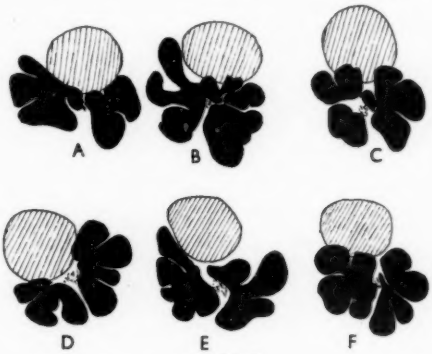


Fig. 5.

Fig. 4. *Ceratotrema furcolabiata.* Shell-gland complex.
Fig. 5. A–F. Vitellaria of six individuals.
OD. oviduct; *OV.* ovary; *R.S.* receptaculum seminis; *S.G.* shell-gland.

situated on the ventral surface of the sinus sac in the mid-line immediately posterior to the termination of the oesophagus.

The vitellaria lie posterior to the ovary and are disposed in two main groups. They exhibit some variation in their arrangement and shape, but more usually there are four main lobes on the right and three on the left. The lobes arise from a common base, are longer than broad, and widen towards their free ends. Fig. 5 A–F represents the variation observed in six specimens.

When the animal is alive a few scattered papillae can be seen round the region of the anterior sucker. These are not visible in stained and mounted preparations and are presumably sensory in function.

Generic Diagnosis. Length 7·3–7·8 mm. Greatest width 1·7 mm. Abdomen completely invaginated into body. Anterior sucker approximately two-thirds size of ventral sucker; average measurements 0·57 and 0·77 mm. respectively. Anterior sucker with three cushion-like projections into lumen, one anterior

and two postero-lateral. Lip dorsally situated, very muscular and strongly bifid, not overhanging the mouth. Ventral groove present. Testes two, oval, situated opposite each other immediately posterior to ventral sucker. Ovary spherical, posterior to testes. Receptaculum seminis present. Vitellaria arranged in two groups posterior to ovary; limbs of vitellaria short and thick and split up to base. Metraterm long, reaching almost to ventral sucker. Eggs green, numerous, $23 \times 11\,\mu$.

Host: *Onos mustela*, in coelom. *Types* in the British Museum (Nat. Hist.).

Systematic position. The species on account of its large size, presence of a distinct abdomen, form of excretory vesicle, naked body, form and position of suckers, gonads and vitellaria belongs to the family Hemiuridae. The presence of a ventral groove, anterior lip, well-defined metraterm and the form of the seminal vesicle and vitellaria place it in the sub-family Sterrhurinae Looss 1907. The genus to which it is most closely allied is *Lecithochirium*, a comparison with which is appended.

	Jajonetta n.g.	*Lecithochirium* Lühe 1901 s.str.
Size	7·3–7·8 mm.	2·5–2·7 mm. (*L. gravidum*)
Lip	Forked, narrow, not overhanging mouth	Wide, rounded, quadrangular, overhanging mouth
Ventral groove	A pit	Transverse groove
Anterior sucker	Three projections into lumen, one anterior, two postero lateral	Two lateral projections into lumen
Testes	Oval, directly opposite each other	Rounded, not opposite each other

Hemipera sharpei n.sp.*

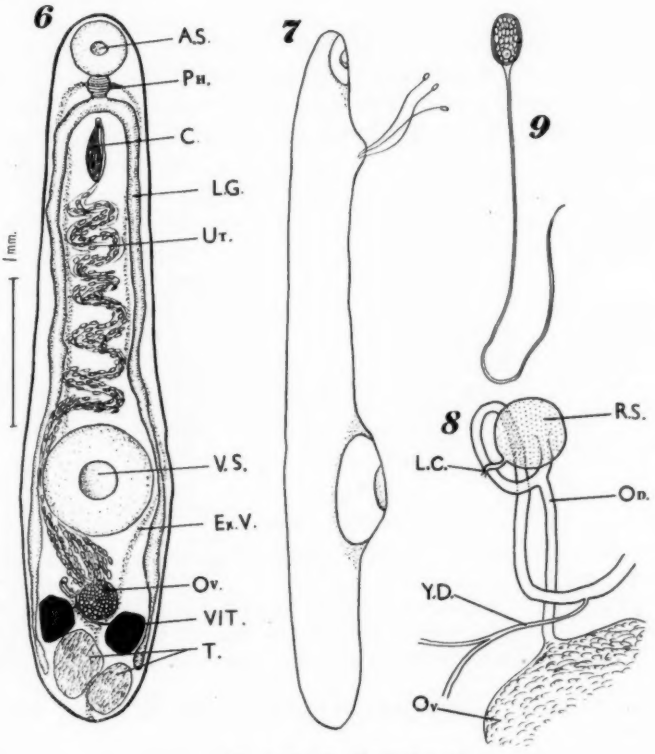
The specimen on which the description of this species is based was found under the gill-cover of *Cepola rubescens* trawled from 30 fathoms at Plymouth. Twelve specimens of *C. rubescens* were examined but only one contained the parasite. It resembles Nicoll's *Hemipera ovocaudata* Nicoll, 1913, but various differences justify its separation as a new species (Figs. 6 and 7).

In size it is considerably larger than *H. ovocaudata* having a length when expanded of 4·77 mm. and 0·85 mm. greatest width. The specimen was mature, eggs being observed escaping from the genital opening when it was removed from its host. It has an elongated form, bluntly rounded posteriorly. The anterior sucker is sub-terminal and 0·372 mm. in diameter. The ventral sucker is situated far back in the posterior third of the body and measures 0·744 mm. in diameter, being thus exactly twice the size of the anterior sucker. There is no prepharynx and the pharynx is small and globular measuring 0·116 mm. in diameter. The oesophagus is very short, the two limbs of the gut arising almost directly from the pharynx and passing down to the level of the posterior testis.

The excretory system. The short common stem of the vesicle bifurcates posterior to the ovary and the two limbs pass up on either side of the body and unite dorsal to the pharynx.

* Named in honour of my teacher Mr W. H. Leigh-Sharpe.

In the posterior region of the body are the *genitalia* which consist of two rounded testes situated one slightly in front of the other, paired vitellaria anterior to the testes and a rounded ovary anterior to the vitellaria. The measurements of these organs are: testes 0·24 mm., vitellaria 0·192 mm., ovary 0·144 mm. There is a small globular receptaculum seminis to the right



Figs. 6–9. *Hemipera sharpei.*

Fig. 6. Ventral view.
Fig. 7. Lateral view.
Fig. 8. Shell-gland complex.
Fig. 9. Egg.

A.S. anterior sucker; *C.* Cirrus sac; *Ex.V.* excretory vesicle; *L.C.* Laurer's canal; *L.G.* limb of gut; *OD.* oviduct; *OV.* ovary; *PH.* pharynx; *R.S.* receptaculum seminis; *T.* testis; *UT.* uterus; *VIT.* vitellaria; *V.S.* ventral sucker; *Y.D.* yolk duct.

of the ovary and a short Laurer's canal leading from it (Fig. 8). The oviduct passes from the ovary to the receptaculum seminis and then bends sharply posteriorly, receiving the common vitelline duct and continuing as the uterus. The uterus is a much coiled structure slightly overlapping the ovary, but no coils pass posterior to it. The middle portion of the body is almost completely occupied by the egg-filled uterus. The uterus terminates at the genital

opening which, in the specimen observed, was situated on a prominent papilla just posterior to the pharynx in the middle line. The cirrus sac is short and muscular, but no details of its structure could be made out. It receives the two vasa deferentia at its posterior end. The eggs are oval in shape, not curved, and each is provided with a very long filament at the anopercular pole. The average measurements of an egg are $100 \times 38\mu$. The filaments of the eggs do not intertwine (Fig. 9).

A few scattered sensory papillae resembling blunt spines are present in the region of the anterior sucker. These are irregularly disposed and are not visible in the mounted preparation. Type in the British Museum (Nat. Hist.).

Table I gives a comparison between the two species:

Table I.

	Hemipera ovocaudata Nicoll 1913	*Hemipera sharpei* n.sp.
Host	*Lepadogaster gouanii*	*Cepola rubescens*
Situation	Stomach	Under gill-cover
	Small and delicate	Large and robust
	Pointed ends	Bluntly rounded
Length	1·54 × 0·56 mm.	4·77 × 0·85 mm.
Anterior sucker	0·22 mm.	0·372 mm.
Ventral sucker	0·40 mm.	0·744 mm.
	Laurer's canal absent and receptaculum seminis not distinct. Eggs slightly curved with short filaments intertwining	Laurer's canal present and prominent receptaculum seminis. Eggs not curved and with long filaments not intertwining
	Egg measures $100 \times 27\mu$	Egg measures $100 \times 38\mu$

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THE HIRUDINEA OF PLYMOUTH. PART I.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 6 Figures in the Text.)

The following marine Ichthyobdellid leeches have been taken at Plymouth:

I. **Calliobdella lophii** van Beneden and Hesse, on a large *Lophius piscatorius*; 1 capture, 4 specimens (*vide* Leigh-Sharpe, 1913 and 1914).

II. **Pontobdella muricata** (Linn.) on various species of *Raia*; rare at times; moderate-sized specimens common on skates and rays in the spring and summer of 1932.

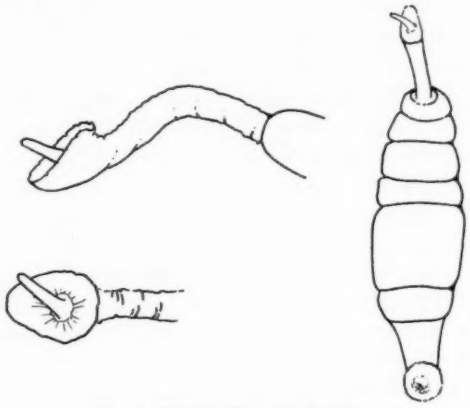


Fig. 1. Presumed young leeches.

III. By the courtesy of Mr G. A. Steven I have been able to examine (Fig. 1) one of two similar small leeches captured by him. The first, which was spoilt in mounting, was taken on 22. i. 1932, on a *Raia maculata* ♂ measuring 29·5 cm. across the disc; and the second on 6. ii. 1932, on a *Raia microcellata* ♀, 60·5 cm. across the disc. In each case the leech was attached to the ventral surface of the body at the edge of the branchial aperture. The second leech was a translucent blood colour by transmitted light; the body brownish red by reflected light. The neck and posterior sucker were white with faint touches of pale orange. The total length of the preserved specimen is 12 mm. I can only suggest that these are the young forms of some leech. The hood-like anterior sucker, the extreme contrast between neck and abdomen, and the preputial arrangement are all suggestive of *Branchellion*.

IV. **JANUSION** nov.gen.

Janusion scorpii (Malm 1860).

Material and habitat. Three specimens of this leech have been taken by me during the past ten years in the Plymouth area, and the one from which Fig. 2 is drawn is from a *Cottus scorpius* trawled in Cawsand Bay on 16. v. 1932. As far as one can judge from the works of ancient authors this is the *Platybdella scorpii* described by Malm (1860). This author, however, attributes it to Fabricius, who, in his *Fauna of Greenland*, p. 321, describes a leech under the name of *Hirudo piscium* which Malm thinks is the same. The only apparent reason for this conjecture is that Fabricius' leech occurred on *C. scorpius*; but we now know that several genera and species of leech have been recorded from this host. In the last century most marine leeches were placed in the genera *Piscicola*, *Ichthyobdella* and especially the genus *Platybdella*, of which the generic diagnoses were so elastic and nebulous that many species have been removed from them to newly founded genera, and the diagnoses of these genera considerably restricted and amended. I have already discussed this question in *Platybdella anarrhichae* (Leigh-Sharpe, 1916, p. 275 footnote), and the leech under consideration so little resembles *Platybdella* as there defined that I have established for it the new genus *Janusion*[1].

Body pale, almost white, with variable greenish markings; cylindrical, only moderately depressed, divided distinctly into a neck and abdomen. When at rest the animal curls itself towards the dorsal surface. Abdomen twice the width of the neck. Two pairs of eyes which under a lens do not resemble the eyes usually found in leeches, but appear as clusters of black pigment spots as seen in some Polychaeta. Annuli of neck and abdomen of much the same size. Total length of preserved specimen, which is much contracted, 12 mm. of which the neck occupies 3·7 mm.

Suckers. Anterior sucker cupuliform, slightly wider than the contracted neck. The two pairs of eyes are situated on the dorsal side of this sucker, the posterior pair being further apart than the anterior. Posterior sucker cupuliform and scarcely twice the width of the anterior and only three-quarters the width of the abdomen at its broadest part. Margins entire.

Segmentation and annulation (Fig. 2).

(*a*) *Anterior sucker.* **5** segments.

(*b*) *Three small annuli* present in most Ichthyobdellid leeches, constituting segment VI[2]. **1** segment.

(*c*) *Preclitellum* nine annuli, of which the eighth is markedly the largest. **3** segments.

(*d*) *Clitellum.* **3** segments.

[1] The two-faced *Janus* would possess four eyes.

[2] An error was made on this point in *Calliobdella* (Leigh-Sharpe, 1914, p. 207).

The first segment is divided into five small annuli; the other two segments are each divided into two annuli; the ♂ and ♀ genital apertures are between the annuli of these two segments respectively.

(*e*) *Abdomen.* **12** segments.

Each segment is divided into two annuli.

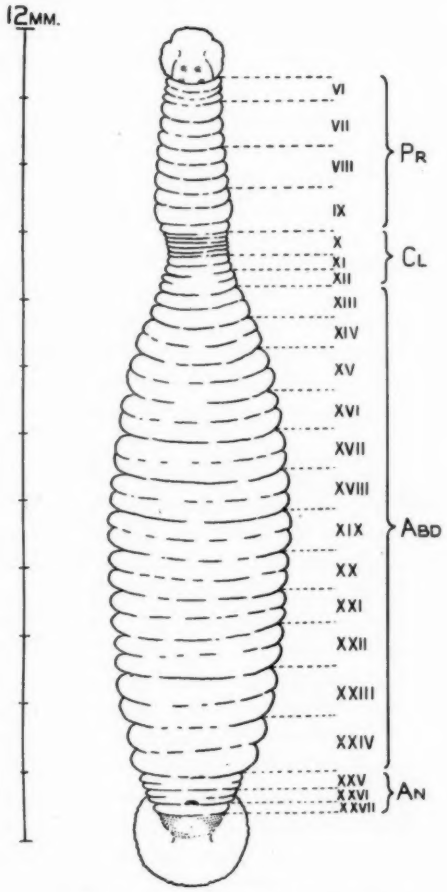


Fig. 2. *Janusion scorpii* in dorsal aspect.

(*f*) *Anal region.* **3** segments.

The first two segments are divided into two annuli, and the last consists of one annulus. The anus is between the last segment and the last but one.

(*g*) *Posterior sucker.* **7** segments.

Total **34** segments.

Generic characters. Body cylindrical, moderately depressed, without papillae or respiratory vesicles, divided distinctly into a neck and abdomen. The leech coils up when at rest. Both suckers cupuliform, entire, the anterior as wide as the neck in the contracted condition. Abdominal segment typically of two annuli (provided that this is a fully grown leech). Two pairs of eyes made up of areas of black pigment spots.

Specific characters. Anterior sucker large in proportion to diameter of neck; posterior sucker three-quarters as broad as the abdomen in the contracted condition. First segment of clitellum divided into five small annuli. Host: *Cottus scorpius*.

Remarks. This species is not to be confused with *Platybdella quadrioculata* which also has four eyes, but does not so closely resemble my specimens as Malm's figure of *P. scorpii*; nor with *P. brunneus*, *P. microstomus* (which Johansson, 1896, has removed to the genus *Abranchus*) and other species which have been found on *Cottus scorpius*.

The genus *Platybdella* as exemplified by *P. anarrhichae*, amended by me in 1916, differs from the genus *Janusion* in having the skin so smooth and the annuli so innumerable and small, that it is impossible to count them and to determine how many annuli constitute a typical abdominal segment. The leech does not roll itself up when at rest. The constriction demarcating the neck from the abdomen is only very slight. There are no eyes.

V. **Branchellion borealis** n.sp.

Material and habitat. My best thanks are due to Mr G. A. Steven for kindly presenting me with a fine specimen of *Branchellion* taken by him on 22. i. 1932, 3½ miles south-west of Stoke Point, on the dorsal surface of the pelvic fin of a *Raia clavata* ♀ 61·5 cm. across the disc.

Branchiae. *Branchellion torpedinis* Savigny was originally described as having 35 pairs of gills, a number which no subsequent collector has observed.

B. orbinensis de Quatrefages was described as having 33 pairs of gills, and that is the number usually found. My astonishment was great, therefore, when I discovered that this Plymouth specimen has 31 pairs of gills, agreeing with *B. australis* which I described from South Australia (1916 *a*), and perhaps with *B. rhombi* van Beneden and Hesse. Moreover its anterior sucker is circular, like that of the two former species, and not hood-like as in the two latter. I accordingly propose the name *Branchellion borealis* for this new species.

Body. Neck very distinctly marked off from the abdomen, with the clitellum partially hidden by a preputial fold. Total length 29 mm., of which the neck occupies 4·2 mm., both measurements exclusive of the suckers. Both suckers cupuliform, the anterior one dark, like the neck, and about twice its width, with its diameter two-thirds that of the posterior sucker, which is white. Neck much more slender than in *B. orbinensis* and *B. torpedinis*. The anterior sucker is conchoidal and on the two more prominent dorsal curves bears five

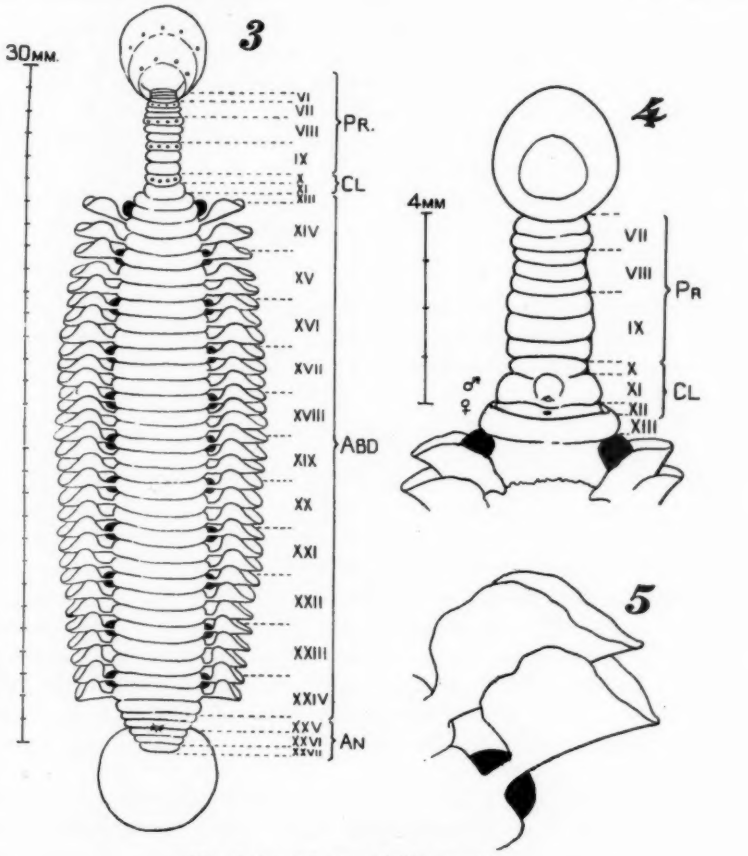
and four light spots respectively. There are no eyes visible in the preserved specimen.

Segmentation and annulation (Figs. 3 and 4).

(*a*) *Anterior sucker:* included in this is one small annulus. **5** segments.

(*b*) *Three small annuli* constituting segment VI. **1** segment.

(*c*) *Preclitellum,* nine annuli. **3** segments.



Figs. 3, 4 and 5. *Branchellion borealis.*

Fig. 3, indorsal aspect; Fig. 4, enlarged view of anterior end in ventral aspect; Fig. 5, enlarged view of a respiratory vesicle and gills.

The first annulus of each segment bears dorsally a row of four small warts.

(*d*) *Clitellum.* **3** segments.

The first segment resembles those of the preclitellum and consists of one annulus bearing four small warts dorsally.

The second segment is clear white, much inflated on each side, consists of one annulus and carries the male genital aperture.

The third segment is smooth, not white, alone covered by the prepuce, bears the female genital aperture, and is doubtfully divided into two annuli.

(*e*) *Abdomen.* **12** segments.

Each segment is composed of three annuli, except the first, which acts as a prepuce and is divided into two annuli only (segment XIII). The remaining 11 segments each bear a pair of respiratory vesicles, one on each side, bordering the first annulus of the segment. In *B. orbinensis* every annulus of these segments bears a pair of lateral foliaceous branchiae, making 33 pairs in all, but in *B. borealis* the first annulus of segment two of the abdomen, which bears a large vesicle, does *not* bear a gill, and the third annulus of the twelfth segment is also without gills, making 31 pairs. Thus the gills corresponding to the first and last pairs present in *B. orbinensis* are absent in *B. borealis*. This gill arrangement is the same as in *B. australis*.

(*f*) *Anal region.* **3** segments.

The first two segments are composed of two annuli each, and the third of one annulus, making five in all. The anus opens dorsally between segments I and II—*i.e.* between annuli 2 and 3.

(*g*) *Posterior sucker.* **7** segments.

Total **34** segments.

Specific characters. Anterior sucker cupuliform, slightly conchoidal dorsally, with rows of five and four white spots; diameter two-thirds of the posterior sucker, which is white and cupuliform. The first annulus of each of the triannulate segments of the neck covered with rows of four warts dorsally, as also is the first annulus of the clitellum. Segment XI, the second of the clitellum, very white and striking, being much inflated laterally, bearing a large male genital aperture, not covered by the prepuce, and consisting of only one annulus. Gills 31 pairs, disposed as in *B. australis*, the first and last of *B. orbinensis* being absent. Eleven pairs of lateral vesicles on the abdomen. No longitudinal rows of six white spots on the first annulus of each abdominal segment. Eyes absent.

I append (Fig. 6) a series of sketches (comparable to Figs. 3, 4 and 5) of *Branchellion orbinensis* de Quatrefages from a specimen kindly loaned to me by Dr R. P. Dollfus of Paris, taken from an unknown host at Fedhala in 1924. The six eyes are not visible in this preserved specimen.

Briefly this species differs from *B. borealis* n.sp. in the following particulars: The neck is more robust; abdomen greyish-black dorsally, black pigment dispersed on anterior sucker. No warts on preclitellum. Four longitudinal rows of white spots on the neck on each annulus and six longitudinal rows of white spots on the dorsal and four on the ventral surface of the abdomen, he spots occurring on the first annulus of each segment and dispersedly on

the anterior sucker. Eleven pairs of lateral respiratory vesicles and 33 pairs of gills more foliaceous than in *B. borealis* but not so long in proportion. Each of the three segments X, XI and XII, of the preclitellum, is plainly

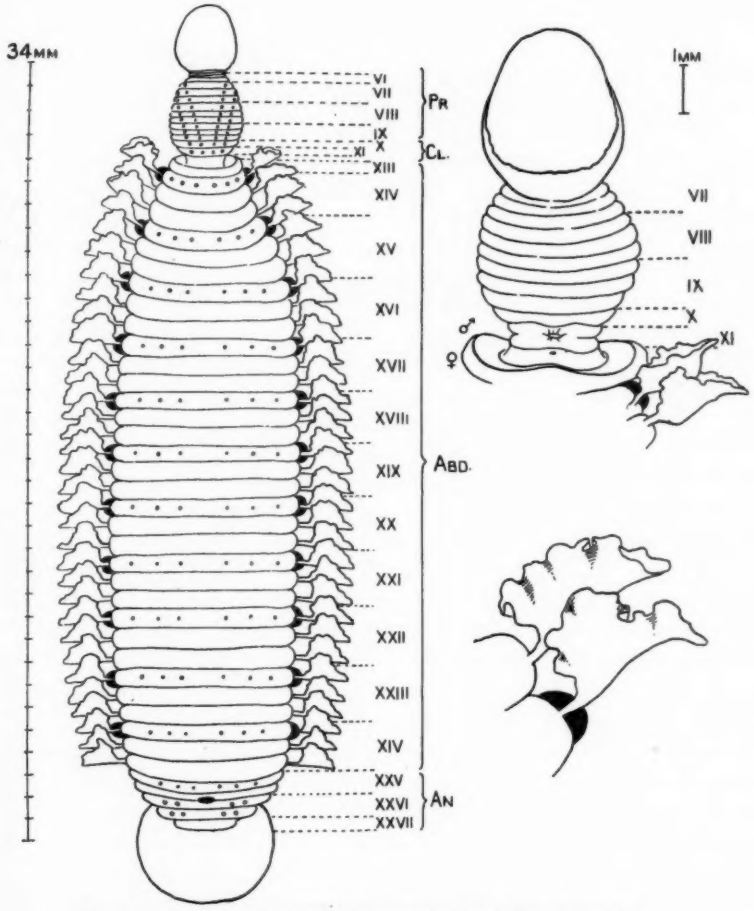


Fig. 6. *Branchellion orbinensis* for comparison with Figs. 3, 4 and 5.

divided into two annuli. The prepuce is much wider. The lateral expansions of segment XI bearing the male genital aperture not so pronounced.

Specimens supposedly of this species are stated to have been taken occasionally at Plymouth, though never on *Torpedo*; not having been carefully examined they have always been assigned to the species *B. torpedinis*.

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Explanation of Lettering on Figures.

Pr. = Preclitellum; *Cl.* = Clitellum; *Abd.* = abdomen; *An.* = anal region; ♂ and ♀ = the respective genital apertures.

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THE ACCELERATION OF DEVELOPMENT OF INSECTS BY PARASITISM.

By G. C. VARLEY, B.A. and C. G. BUTLER.

From the Zoological Laboratory, Cambridge.

Introduction.

It has been known for many years that the presence of a parasite may affect the rate of development of its insect host but, although Pantel (1912) called attention to several cases of both accelerated and retarded development, no general discussion of the subject has yet appeared. The observation by Dr George Salt that the parasite *Polemon liparae* causes premature pupation of its host, *Lipara lucens*, and by one of us (G. C. V.) that *Eurytoma curta* has the same effect upon *Urophora solstitialis* aroused our interest in the matter, and in the following pages, besides presenting new data, we have attempted to bring together and discuss all that is at present known about the acceleration of development by parasitism. This is a problem which can be finally elucidated only by experimental analysis; but we hope that the present notes, by clearing the ground and attracting attention to the subject, will bring its solution nearer.

We are greatly indebted to Dr George Salt for suggesting the problem to us, and for much assistance in gathering our data and preparing this paper for publication. We also wish to thank Dr Ch. Ferrière for identifying our parasite material.

Review of the literature.

Even before the middle of the last century it had been noted (Westwood, 1840, p. 300) that stylopised specimens of a species of *Andrena* appeared earlier than the normal individuals. A similar precocity of stylopised individuals of *Prosopis rubicola* was reported by Saunders (1850), who found stylopised pupae in May, when unparasitised specimens were still larvae. Pierce (1909, p. 30) published field data showing that stylopised *Andrena crawfordi* appeared before unstylopised individuals, and Perkins (1918) gave more exact notes showing that the percentage of stylopised individuals of *A. wilkella* fell from 100 per cent. on April 27th to 90 per cent. on May 4th, 30 per cent. on May 7th, and less than 10 per cent. on May 11th. Salt (1927, p. 276) mentioned that the same gradual decline in the percentage of stylopisation occurs in *A. hirticincta* and in several species of *Chloralictus*.

Stylopisation, however, is not the only form of parasitism that causes acceleration of development. Pantel (1912, p. 123) mentioned three cases of accelerated development, two of Lepidoptera and one of Coleoptera, due to

infestation by Tachinidae. Larvae of *Chondrostega vandalicia* infested by larvae of *Uclesia fumipennis* ceased feeding before their unparasitised fellows and prematurely commenced to spin their cocoons; *Acronycta aceris* behaved similarly when parasitised by *Compsilura concinnata*, and *Crioceris* sp. when attacked by *Meigenia floralis*.

Altson (1920) stated that the larvae of *Calliphora erythrocephala* attacked by the Braconid parasite *Alysia manducator* were stimulated to immediate pupation. Holdaway and Evans (1930) found that larvae of *Lucilia sericata* collected at baits during the late autumn and winter months were unparasitised[1], while puparia from the same source showed a parasitism of 87–90 per cent. by *Alysia manducator* and *Aphaereta minuta*, the remainder being unparasitised. Salt (1932) reported that among some thousands of reared individuals of *Lucilia sericata* the unparasitised maggots entered the usual winter diapause while the parasitised larvae pupated. Salt also stated that starved half-grown larvae which would normally fail to pupate, might yet under the stimulus of parasitism accomplish pupation.

There are in the literature, then, recorded cases of the accelerated development of Apidae, Lasiocampidae, Noctuidae, Chrysomelidae, and Muscidae by parasites of the families Stylopidae, Tachinidae and Braconidae. Before discussing the phenomenon in a general way we shall describe two additional cases which we have ourselves studied, and which add two families, Chloropidae and Trypetidae, to the list of hosts, and one, Chalcididae, to the list of parasites.

The effect of *Polemon liparae* on *Lipara lucens*.

Lipara lucens Meigen is a Chloropid fly that forms galls on the stems of the common reed, *Phragmites communis*. The larva is full grown in autumn, but under normal circumstances hibernates in the last larval instar and does not pupate until the following May. During the months December, 1931 to April, 1932, one of us (C. G. B.) examined 1052 larvae of *L. lucens* taken from their galls in the reeds: 150 of them were dissected, 607 emerged, and 295 died, but not one of them was found to be parasitised. From field observations and the laboratory experiments to be described below it is certain that these larvae were in an obligatory diapause and would not have pupated until May.

On the other hand, in fifty cases during the same period the gall on being cut open was found to contain a puparium of *L. lucens* instead of a larva. In every case the puparium contained a larva of the Braconid parasite *Polemon liparae* Giraud.

It cannot be doubted, then, that individuals of *L. lucens* which would normally have entered a diapause and hibernated as larvae, had in some way been stimulated by parasitism to pupate in autumn, some six months before the normal time.

[1] These authors do indeed say that *Alysia* was bred from seven larvae which had not pupated eight days after collection, but, as *Alysia* can only emerge from a puparium, it may be assumed that these pupated later.

THE EFFECT OF *EURYTOMA CURTA* ON *UROPHORA SOLSTITIALIS*.

The larvae of the Trypetid fly *Urophora solstitialis* Linn. form multilocular galls in the flower heads of the knapweed *Centaurea nigra* Linn. (Wadsworth, 1914). Each larva feeds in a separate flask-shaped cell with its dark sclerotised anal plate directed upwards towards the opening of the cell. The larva remains in this position throughout the winter when, as shown by experiment, it is in an obligatory diapause. In May the larva first reverses its position in the cell so that its head is directed towards the aperture, and then forms its brown puparium and pupates. From January to April, 1932, 487 living larvae of *U. solstitialis* were obtained near Cambridge; twenty-two died, and the rest emerged as adults. Not one was parasitised.

During the same period 146 puparia of *U. solstitialis* were found, and every one of them contained a parasite larva. Fourteen died, two were preserved and 130 emerged as imagines of the Chalcidid *Eurytoma curta* Walker. From a single observation of an ovipositing female it appears that the egg is placed within the first-instar larva of the host about the end of July; and from several observations it is certain that after feeding to approximately full size the parasitised hosts turn to face the aperture of the cell and form their puparia in September. It is clear, then, that when *U. solstitialis* is parasitised by *E. curta* it is caused to pupate eight months before the normal time.

DISCUSSION.

Two points are common to all the known cases of accelerated development due to parasitism. First, in every case the parasite producing the effect is endophagous. This is not surprising, for an endophagous parasite is in a far more intimate relation with its host than an ectophagous form, and may directly affect it by abstraction of nourishment from the tissues, or by liberating substances into the body fluid. Secondly, in all cases in which the life history of the host has been observed, the acceleration of development first manifests itself in the precocious onset of pupation. This may be significant as an effect on the duration of the larval stage, or it may be only apparent, and actually due to the greater ease of observing the definite changes associated with pupation.

Apart from these two points which they have in common, the examples to be considered in this discussion fall naturally into three groups: (1) Pantel's cases, in which the pupal stage is not actually reached; (2) cases in the Diptera, in which the pupal stage is just attained; and (3) cases of stylopisation, in which pupation is successfully completed and followed by emergence. It will be most convenient to discuss each group separately.

Pantel's data are too meagre to lead to definite conclusions. His examples all refer to phytophagous hosts parasitised by Tachinid larvae. It should be noted also that *Compsilura concinnata*, which accelerates the development of

Acronycta, is said by Pantel to retard the development of *Vanessa*. Beyond these points comment is useless until further information is available.

In the cases of stylopisation the data are again scanty. For *Andrena* and *Chloralictus* we have only the fact that stylopised adults appear prematurely without any information about the actual stage which is accelerated. In *Prosopis rubicola* stylopisation is known to produce premature pupation as well as early emergence. On the other hand, Wheeler (1910, p. 393) has shown that when *Polistes* is stylopised pupation is retarded, the feeding period of the host being prolonged. Salt (1927) noticed that the morphological effects of stylopisation may be correlated with the feeding habits of the hosts, and he pointed out that the acceleration or retardation of the hosts may also be related to their method of feeding, since *Andrena* which is accelerated has a fixed ration of food, while *Polistes* which is retarded is a progressive feeder, and can continue to feed until satisfied. The mechanism involved here, however, needs further study.

Finally we come to the cases in the Diptera, in which the pupal stage is just attained. The evidence at our disposal here is much more complete and consists in part of experimental work, and it enables us to come to some definite conclusions. The parasites in these cases are all Hymenoptera which induce their dipterous hosts to pupate in autumn instead of hibernating as larvae. This fact immediately calls to mind the work of Roubaud on hibernation in the Muscidae.

Roubaud (1922) carried out experiments on the hibernation of certain flies. He found that in autumn the fully grown larvae of several Diptera, including *Lucilia sericata*, enter a state of hibernation, the diapause, in which they remain without pupating even if placed at a temperature ordinarily suitable for continued development. He found, further, that if the larvae are subjected to a period of cold the diapause is broken and pupation ensues. Most important for our present purpose, however, he found that in some species, especially *L. sericata*, certain types of shock—singeing, pricking, centrifuging, and sudden changes in temperature—also break the diapause and allow the larvae to pupate. It is our opinion that these results are closely related to the present problem.

The work of Roubaud actually dealt with one of the species under discussion, *L. sericata*, but, in order to relate his experimental results to one of our own species, one of us (C. G. B.), carried out the following experiments on *Lipara lucens*.

I. On December 3rd, 1931, thirty-seven galls of *Lipara lucens* were placed in an incubator at 25° C. and 80 per cent. relative humidity. After ten months, no flies having emerged in the meantime, they were cut open. They were found to contain thirty-seven dead grubs, not one of which had pupated. The full-grown larvae collected early in December, then, were in an obligatory diapause.

II. One hundred and twenty larvae collected on December 5th were re-

moved from their galls, isolated in gelatine capsules, and placed in a refrigerator at 0° C. After ten days they were removed and placed in the incubator. Thirty-one pupated in the first three days, twenty-seven on the fourth day, five on the fifth day, nineteen on the sixth day and nine on the seventh day; the remaining twenty-nine died without pupating. Of the ninety-one which pupated, seventy-eight emerged, the remainder dying in the pupal stage. A number of controls placed directly in the same incubator failed to pupate and eventually died as larvae. This experiment shows that a period of cold will break the diapause and allow development to proceed.

III. Fifty larvae collected on December 3rd were submitted to shocks; twenty were singed with a red-hot needle; twenty were pricked with a needle dipped in 5 per cent. acetic acid; and ten were given tetanising induction shocks with a Du Bois Reymond induction coil. They were then placed in the incubator with an equal number of control larvae. Of the singed larvae eleven, of the pricked larvae two, and of the tetanised larvae four pupated within seven days. None of the controls pupated. It is apparent, then, that a violent stimulus will also break the diapause and cause premature pupation.

In brief, then, we have these facts: that *Lucilia sericata*, *Lipara lucens*, and *Urophora solstitialis* normally hibernate as larvae; that certain experimental stimuli will induce the first two to pupate; that natural parasitisation by *Alysia manducator*, *Polemon liparae*, and *Eurytoma curta*, respectively, will induce all three to pupate. It is strongly suggested that parasitisation provides the stimulus necessary to break the diapause. This, indeed, is our main thesis—that the acceleration of development by parasitism in the case of these flies is merely a special and natural case of the phenomenon studied experimentally by Roubaud.

When we come to examine the nature of the stimulus concerned in each case we find a difference. In the case of *Lucilia sericata* it is probable that the stimulus is afforded by the sting of the parasite. Myers (1927) has described the attack of *Alysia manducator* on *Lucilia sericata*. As soon as the maggot is stung its violent struggles cease and it lies for a time completely paralysed, even the heart ceasing to beat for some minutes. There is no doubt, then, that the host receives a shock at least as violent as the experimental singeing and pricking. Salt (1932) has shown that *Alysia manducator* sometimes paralyses its host without actually ovipositing in it, which would explain, if the sting were the stimulus, why Holdaway and Evans (1930) found some unparasitised puparia of *Lucilia* among hibernating individuals that had been exposed to parasites, while Davies (1930) found no winter puparia in an unparasitised stock. All these facts point to the conclusion, also reached by Holdaway and Evans on their data alone, that it is the shock of the sting of *Alysia* that causes premature pupation in *Lucilia sericata*.

In the case of *Lipara lucens* and *Urophora solstitialis*, on the other hand, there is no evidence of a shock on oviposition, which, moreover, probably occurs in both cases long before pupation takes place. These two cases also

differ from that of *Lucilia* in that all the winter puparia are parasitised. It is probable, therefore, that these larvae are induced to pupate by some effect, as yet obscure, of the parasite larva within them, rather than by any shock given by the ovipositing adult parasite.

SUMMARY.

1. Cases of accelerated development of insects due to parasitism are reviewed.

2. Two new cases are recorded.

3. The phenomenon is discussed in the light of Roubaud's study of the diapause.

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RHIPICEPHALUS AYREI N.SP. (A TICK) FROM KENYA COLONY.

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Entomologist, Veterinary Research Laboratory, Kabete.

(With 2 Figures in the Text.)

Male: Large, 4·00–7·75 mm. long. *Salient features*: Inornate; basis capituli nearly twice as broad as long; lateral angles within the anterior third and rather acute. Anterior coxae slightly prominent in dorsal view. Lateral grooves well-marked and including two festoons. Dorsal furrows distinct, the lateral somewhat comma-shaped, the median straight and continued anteriorly beyond the laterals. Punctations very numerous, usually discreet and equal in the median area which appears shagreened; few on the scapulae, lateral folds and festoons, which form a glossy border. Adanal plates with straight external border and broad, rounded posterior border; internal border slightly concave to where it joins the posterior border to form a small point in a number of specimens. Accessory plates distinct. Spiracle short with slight dorsal curve.

Detailed description. Capitulum: Palps fairly long, concave dorsally, margins angular; article 3 a little shorter than 2; article 1 visible, but inconspicuous in dorsal aspect. Basis capituli broader than long; anterior border nearly straight, antero-lateral in most specimens interrupted by a collar-like groove; postero-lateral border concave and posterior nearly straight and provided with strong, short cornua. No auriculae. Median field of basis depressed, margins raised and glossy.

Scutum 4·8 × 3·5 mm. in the largest specimen, 3·0 × 2·0 mm. in the smallest. Punctations numerous, deep, usually discreet, but sometimes contiguous particularly in larger males; of uniform size in median field; more unequal in size and in distribution in the humeral regions, whereas on the folds external to the lateral grooves they are mainly fine and sparsely distributed. Cervical grooves short, deep pits generally continued by short, more shallow diverging depressions. Lateral grooves well-defined, but not deep except towards the posterior end where they include the anterior pairs of festoons; they are formed by a number of unequal pits. Eyes flat, yellowish.

Body: External festoons broader than long, the internal and most posterior festoons longer than broad; posterior festoons with coarse punctations anteriorly. Dorsal grooves (as in Fig. 1) variable in length; median groove may be longer, the laterals may be somewhat broader and shorter than figured. Body dark brown dorsally to beyond scutum in distended specimens.

Most males have a thick, blunt caudal process with a dark-brown basal plaque. In well-fed males, a conspicuous protuberance of body contour opposite the three lateral festoons; second festoon attaining a dome-like appearance.

Venter: Legs light chocolate-brown, contrasting with much darker scutum. Coxal armature normal. Adanal plates as described above with a few coarse and fine punctations; accessory plates bluntly pointed, with few punctations. Spiracle as in Fig. 1.

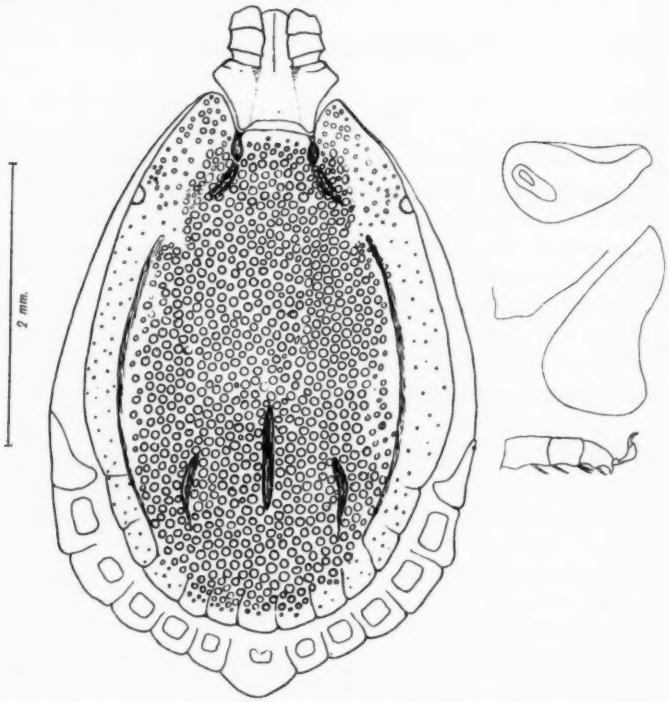

Fig. 1. *Rhipicephalus ayrei* n.sp. ♂, dorsal aspect. Spiracle, adanal plates of right side, tarsus.

Female. All the specimens examined were large, *unfed*; one of average size: 7·54 × 5·00 mm., has a scutum measuring 2·19 × 2·27 mm. Although resembling *R. sculptus* in most respects, sculptural markings are absent. *Scutum* slightly broader than long or rounded with a depressed roughly shagreened central area strongly pitted with deep, equal, often contiguous punctations; lateral borders raised, glossy, with some fine and a few large punctations; cervical grooves short, deep, followed by faint, diverging furrows which disappear at the level of the eyes. (The raised punctate island present in the region between the cervical grooves and the lateral ridges of *R. sculptus* is very faintly marked in some specimens of *R. ayrei*, and appears to be

absent in the majority). Palps long, without a distinct dorsal concavity. *Basis capituli* clearly broader than long, lateral angles situated anteriorly, external borders raised and separated from the median hollow by a definite ridge; porose areas rounded, with interspace wider than their diameter, a short anterior gutter frequently leads to the porous areas; posterior margin of basis straight, cornua distinct.

Body: dorsal furrows as in *R. sculptus*. Punctations large, sparse, from these and the grooves arise short, white, glistening hairs. Festoons as in male; lateral festoons broader than long, posterior festoons longer than broad. Spiracle short, with dorsal curve.

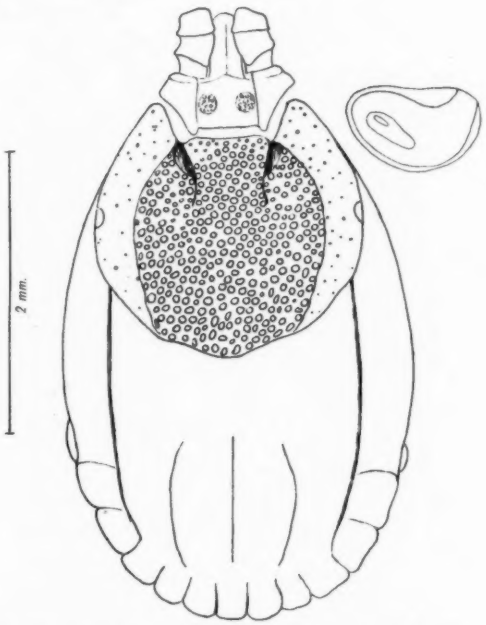

Fig. 2. *Rhipicephalus ayrei* n.sp. ♀, dorsal aspect (unfed), spiracle.

Species described from a number of ♂♂ and ♀♀ specimens; 10 ♂♂ and 20 ♀♀ were taken from a buffalo at Kiagu, Meru district on 22. x. 1930, 16 ♂♂ and 20 ♀♀, along with many *R. supertritus*, from a rhinoceros near Mount Kenya on 29. x. 1930, 4 ♂♂ and 11 ♀♀ from a buffalo (abdomen, groin), at Mbeyo, Meru district on 29. x. 1930, and 3 ♂♂ and 1 ♀ from a lion on the Sianna Plains, Masai Reserve on 10. i. 1931.

The adanal plates of the male sufficiently distinguish it from *R. supertritus* Nn. 1907. In general appearance it greatly resembles *R. sculptus* Warburton 1912, but the numerous specimens we have examined are all

devoid of the characteristic sculpture of that species. The species is named after Mr A. F. Ayre, a well-known game hunter, and a keen naturalist, in Kenya Colony. Many specimens have been forwarded to me from time to time by Mr Ayre, and I am also indebted to him for other interesting specimens.

Cotypes of *R. ayrei* have been forwarded to the Imperial Bureau of Entomology, British Museum (Natural History) and to The Molteno Institute for Research in Parasitology, University of Cambridge. A collection has been retained at the Veterinary Research Laboratory, Kabete, Kenya Colony, and a collection has been sent to the Coryndon Memorial Natural History Museum, Nairobi, Kenya Colony.

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STUDIES IN TICK-BORNE FEVER OF SHEEP.

I. TRANSMISSION BY THE TICK, *IXODES RICINUS*, WITH A DESCRIPTION OF THE DISEASE PRODUCED.

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From the Moredun Research Institute, Edinburgh.

(With 4 Temperature Charts.)

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Introduction.

During the preliminary investigations into the transmission of a disease of sheep known as louping-ill, it was found that unfed nymphs of *Ixodes ricinus* collected from pastures in louping-ill districts were capable of causing an acute febrile reaction in sheep (MacLeod, 1932). This reaction was not considered to be due to louping-ill infection, as after recovery the animals were not immune to louping-ill. It was found also that a febrile reaction immunologically distinct from louping-ill occurred in sheep grazed on a tick-infested farm (Gordon *et al.* 1932 *a*). This febrile condition was further studied, and it has now been shown (Gordon *et al.* 1932 *b*) that the reaction was identical with that produced experimentally by MacLeod. The condition has been named tick-borne fever. When transmitted by inoculation of blood from affected sheep the disease is characterised by an incubation period of about 4 days, followed by a febrile phase which lasts about 10 days. The presence of the infective agent has been demonstrated in the blood, spleen and central nervous system of affected animals. The mortality is low, and after recovery most animals are relatively immune to further infection. In animals killed during the febrile phase the only gross pathological change observed was splenic enlargement.

In the present paper we describe: (1) experiments on the transmission of tick-borne fever by ticks collected from pasture, and by ticks which in their

previous stage had engorged on sheep affected with the disease; (2) experiments in the transmission of the disease by inoculation of infective ticks; (3) the duration of infectivity of the blood of recovered animals; (4) infection of the goat with tick-borne fever; (5) the salient features of the disease, as produced by experimental infestation.

Transmission of tick-borne fever.

The descriptions of the different infestation experiments have been condensed, the information being given in the following order: the stage and number of ticks used; the day on which the temperature reaction (T.R.) began, and the duration of the reaction; the highest temperature recorded. There then follows a note on any further experiments, such as inoculation of blood into a tick-borne fever immune and a control normal sheep, for the purpose of proving that the reaction was due to tick-borne fever, or inoculation of blood into mice in order to test for the presence of louping-ill virus. The latter was carried out as follows: Blood drawn from the reacting sheep was diluted 1 in 5 with citrate solution; mice were inoculated intracerebrally with 0·1 c.c. each of the mixture and kept under observation for the following 21 days. (None of them developed louping-ill, whereas it is known that mice similarly inoculated with blood from a louping-ill case during its febrile reaction develop louping-ill usually in about 6 days (Gordon *et al.* 1932 *a*).)

Transmission by ticks collected from pastures.

Unfed nymphal and female ticks were collected from louping-ill pastures in May. In the following August they were allowed to engorge on healthy blackface tups, the scrotal sac method being used (MacLeod and Gordon, 1932).

Tup 249. About 60 nymphs attached. T.R. on 5th day; lasted 9 days. Highest temperature 107·2° F. (Chart I). Blood tested during the first 6 days of reaction, and louping-ill virus found to be absent.

Tup 245. Sixty-seven nymphs attached. T.R. on 5th day; lasted 15 days. Highest temperature 107·2° F. Louping-ill virus absent from blood tested on 6th day (temperature at this time 107·2° F.). On recovery sheep inoculated with louping-ill virus and found to be non-immune.

Tup 276. Infested with "clean" nymphs from stock bred on a hedgehog, as a control. Heavy infestation. No T.R.

Tup 250. Infested with females. T.R. on 14th day; lasted 5 days. Highest temperature 107° F. Blood tested at peak of reaction (15th day) did not contain louping-ill virus. On recovery sheep inoculated with louping-ill virus and found to be non-immune.

Tup 278. Infested with females. T.R. on 10th day; lasted 6 days. Highest temperature 107° F.

Transmission by ticks which had fed in their previous stage on affected sheep.

Numerous ticks, gorged on various cases of tick-borne fever which occurred in the field[1], were collected throughout the course of the reactions.

[1] For the history of these cases see the account of the 1931 field experiment (Gordon *et al.* 1932 *a*).

This material was bred in the laboratory and furnished ticks of which the histories of the hosts for the previous stage were accurately known. With these a number of infestation experiments were carried out, the results of which are recorded below. The term "infected" is here used to designate ticks which fed on a sheep during the course of its reaction. The "infected" ticks had all moulted to the next stage by the end of June; the infestation experiments were commenced early in August. In the case of the first six infestations the primary object was to secure transmission of the disease; accordingly females and larvae bred respectively from "infected" nymphs and "infected" females were used together. These six experiments were completed in August.

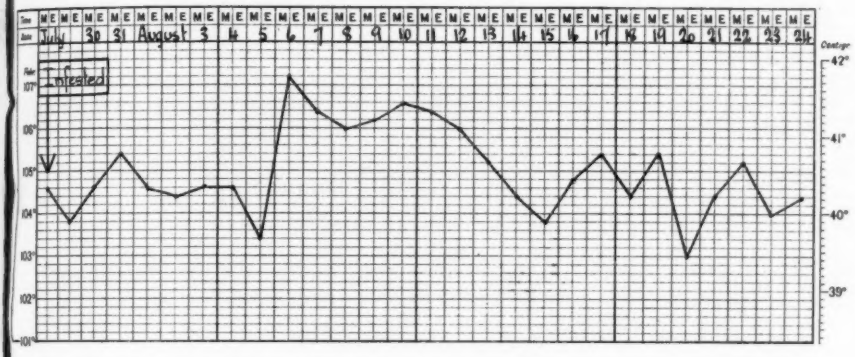

Chart I. Temperature reaction in Sheep 249 following infestation with nymphs collected from pastures.

Females and larvae.

Tup 275. (This lamb was in a debilitated condition.) Two females and numerous larvae attached. T.R. on the 10th day after infestation; highest temperature 105·8° F. The sheep died on the 16th day from bacillary necrosis of the liver.

Tup 277. Four females attached, and numerous larvae. T.R. on the 8th day; highest temperature 107·8° F. Blood was tested each day for the first 4 days of pyrexia and did not contain louping-ill virus. (Nine days after the beginning of the reaction the sheep was used for an experiment on the effect of tick-borne fever on louping-ill infection.)

Tup 246 (Chart II). Nine females attached, and numerous larvae. T.R. on 8th day; lasted for 13 days. Highest temperature 108° F. Blood from this sheep caused no reaction in a tick-borne fever immune sheep, No. 85, and caused a temperature reaction in a normal sheep, No. 293.

Tup 282. Two females and numerous larvae attached. T.R. on 10th day, lasting for 6 days. Highest temperature 107·3° F.

Tup 247. One female and numerous larvae attached. No T.R.

Tup 287. Two females and numerous larvae attached. No T.R. This sheep was subsequently shown by inoculation of infective blood to be susceptible to the disease.

Of the above six sheep infested with females and larvae bred from "infected" ticks, four reacted and two did not react. The explanation of the failure of these two to react is not known; one, at least, was susceptible, as

shown later by inoculatiou. It is to be remembered, however, that they were infested with only one and two infective ticks respectively; the larvae were non-infective, as shown later. With such small numbers of ticks the chances of the sheep becoming infected are reduced.

In the following experiments, which were carried out from the end of August onwards, the infectivity of each stage of the tick was tested separately.

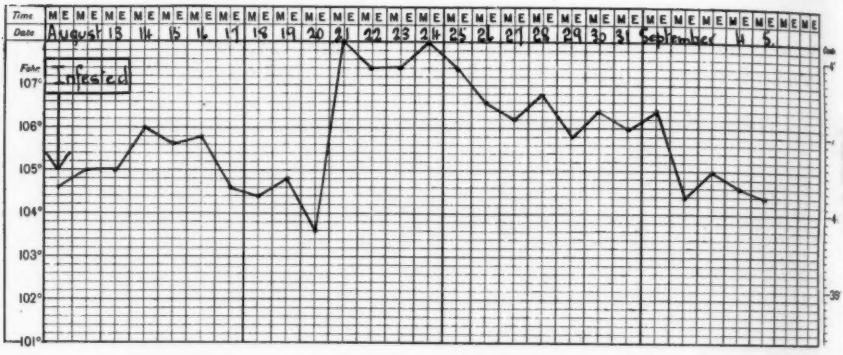

Chart II. Temperature reaction in Sheep 246 following infestation with females which as nymphs gorged on a case of tick-borne fever.

(*a*) *Transmission by females.*

Tup 288. Five females attached. T.R. on the 7th day; lasted for 12 days. Highest temperature, 107·4° F. Blood from this case caused in a normal sheep, No. 306, a T.R. which began on the 4th day and lasted 4 days.

Tup 323. T.R. on 7th day; lasted 11 days. Highest temperature 106·2° F. Louping-ill virus absent from blood tested at peak of reaction (8th day).

Tup 281. Fifteen females attached. T.R. on 8th day. Highest temperature, 107·8° F. Destroyed on 4th day of reaction. Louping-ill virus absent from blood and tissues.

Tup 276. Several females attached. T.R. 7th day; lasted 14 days. Highest temperature 106·8° F. Louping-ill virus absent from blood tested on 9th day. Blood inoculated into a normal sheep, No. 351, caused a T.R. on 5th day.

Tup 447. Several females attached. T.R. 7th day; lasted 22 days. Highest temperature 107·4° F.

Tup 244. Fourteen females attached. T.R. 6th day. Indefinite reaction; temperature did not exceed 105·2° F. Peaks on 6th, 16th and 23rd day.

Tup 500. Six females attached. T.R. 12th day; lasted 10 days. Highest temperature 107·4° F.

Tup 300. Several females attached. No T.R. This sheep was shown by subsequent inoculation with infective blood to possess a relative degree of immunity to the disease.

Tup 448. Several females attached. No T.R.

Tup 594. Five females attached. No T.R.

Of the above ten sheep infested with females bred from "infected" nymphs, six reacted definitely, one gave an indefinite reaction, and three did not react. One non-reactor was subsequently inoculated with infective blood and proved to be relatively immune. The other two were not tested further.

(*b*) *Transmission by nymphs.*

Tup 286. Thirteen nymphs attached. T.R. on 7th day; lasted for 12 days. Highest temperature 107·3° F. (Chart III). Louping-ill virus absent from blood tested on 8th day. Blood inoculated into a normal sheep, No. 308, caused a T.R. on the 4th day.

Tup 456. Six nymphs attached. T.R. 7th day. Highest temperature 107·6° F. Died on 6th day of reaction. No gross lesions which might have caused death. Louping-ill virus absent from blood. Blood inoculated into a normal sheep caused a typical tick-borne fever reaction. Brain and cord examined—no louping-ill lesions or virus present.

Tup 614. Eleven nymphs attached. T.R. on 7th day; lasted 14 days. Highest temperature 107° F.

Tup 615. Thirteen nymphs attached. T.R. on 8th day; lasted 14 days. Highest temperature 107° F.

Tup 244 (control experiment). Infested with nymphs bred from larvae gorged on hedgehog. No T.R.

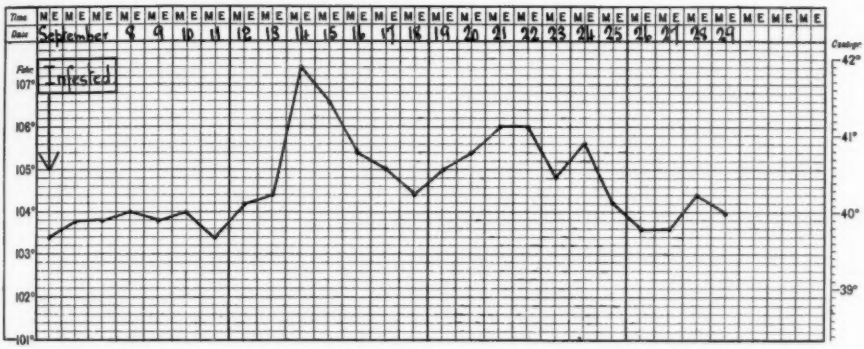

Chart III. Tick-borne fever reaction in Sheep 286 following infestation with nymphs infected as larvae.

(*c*) *Transmission by larvae.*

Eleven tups were infested, each with several hundred larvae bred from "infected" females, and none of them reacted. Eight of these were subsequently shown to be susceptible, four by infestation and four by inoculation; one was not tested further, and two were found to be immune when tested by infestation. In addition to this, there is the following negative evidence obtained before the disease was recognised:

Sheep 911, infested with larvae collected from pastures in an infected area, did not react.

Sheep 983 and 999, infested with larvae—the progeny of females engorged on sheep in an infected area—did not react.

It appears, therefore, that there is no hereditary transmission of tick-borne fever, *i.e.* the infection acquired by the female apparently does not pass through the egg stage of the tick.

Experiments on transmission by emulsions of ticks.

Exp. 1. Females and nymphs at all stages of engorgement were collected from two sheep reacting to tick-borne fever in the field. Fifteen days later these ticks were washed in saline, weighed and emulsified in a sterile mortar with sufficient sterile saline to yield a 1 in 10 dilution. This was centrifuged for 20 min. at about 2000 R.P.M., and the supernatant fluid, in varying dilutions, inoculated intracerebrally into mice. The mice remained healthy.

Exp. 2. A gorged female, collected from a case of tick-borne fever in the field, was allowed to oviposit in the laboratory, and the eggs hatched to larvae. 0·5 g. of these was ground up in sufficient saline to yield a 1 in 10 dilution. This was centrifuged as before and inoculated into mice, all of which remained healthy.

Exp. 3. Nymphs and larvae were allowed to feed on an experimental case of tick-borne fever (Tup 288). After engorgement, they were collected and kept in the laboratory for 24 days. They were then incubated at 30° C. for 24 hours, and the larvae and nymphs separately emulsified. Sufficient saline was added to each emulsion to yield a dilution of 1 in 10. These emulsions were centrifuged, and the supernatant fluid inoculated intracerebrally into mice. Of the mice inoculated with the nymphal emulsion, one died from the operation, one died in 8 days, and two survived. Of the mice inoculated with the larval emulsion, three survived and one died in 8 days. The deaths which occurred are believed to have been due to bacterial contamination.

It has already been shown that infective blood does not produce a reaction when inoculated into mice, thus the failure of the tick emulsions to produce a reaction in mice was to be expected. These results afford further evidence in support of the view that this disease is distinct from louping-ill, for it is known that mice react to intracerebral inoculation of emulsions of ticks infected with the louping-ill virus (MacLeod and Gordon, 1932).

In the following experiments the tick emulsions were inoculated subcutaneously into sheep. The unfed ticks were incubated before being emulsified, since it has been shown in the case of Rocky Mountain Spotted Fever that ticks which readily infect by biting are not regularly infective when emulsified and injected, unless previously incubated (Spencer and Parker, cited by Theiler, 1928).

Exp. 4. Seventeen unfed adults and some 2000 larvae, bred from nymphs and adults gorged on a field case of tick-borne fever, were incubated at 30° C. for 24 hours. They were then emulsified in saline with sterile sand and the emulsion, after removal of the débris by light centrifugalisation, was inoculated subcutaneously into a normal sheep, No. 310. This sheep did not exhibit a thermal reaction, and when subsequently tested with virulent blood was found to have developed no immunity to the disease.

Exp. 5. Gorged nymphs were collected from a number of field cases of tick-borne fever and bred to adults. Some of the females were allowed to feed on a normal sheep, No. 244, to test their infectivity. This sheep developed an indefinite attack of tick-borne fever. The remainder of the unfed ticks, consisting of seventy-three adults, were incubated for 24 hours at 25° C., weighed, and emulsified with sterile sand in enough saline to yield a 1 in 25 dilution. After sedimentation of the sand and heavier particles, the emulsion, amounting to about 4 c.c., was inoculated subcutaneously into a sheep, No. 407. This sheep did not react.

In view of the above negative results, the effect of allowing the tick to become partially engorged on a susceptible animal before inoculation was

tried. It has been shown by Theiler (1928) that preliminary partial engorgement of the tick is necessary in the case of East Coast Fever, and increases the probability of infection in the case of heartwater.

Exp. 6. Of the females which were allowed to feed on Tup 244 seven were removed when partially engorged and emulsified in saline with sterile sand. After sedimentation, the emulsion was inoculated into a normal sheep, No. 415. This sheep did not react.

Exp. 7. Females bred from nymphs gorged on a field case of tick-borne fever were allowed to gorge on a sheep, No. 447, and produced a typical tick-borne fever reaction. Eight days after infestation, when practically fully gorged, these ticks were removed from Sheep 447, and 0·25 g. was emulsified with sterile saline. The emulsion, after sedimentation of the heavier particles, was inoculated subcutaneously into a sheep, No. 481. The sheep did not react.

The failure of the tick emulsions to cause a reaction when inoculated subcutaneously into sheep is of considerable interest. Ticks which were known to be infective by biting failed to cause a reaction, either when emulsified as unfed ticks after incubation, or when emulsified after partial engorgement. That is, in the limited number of experiments carried out, the infective agent in the tick was not rendered virulent either by incubation at 25 or 30° C. or by the ingestion of blood by the tick.

Infectivity of blood of recovered cases.

It has previously been shown (MacLeod, 1932) that the blood of a recovered case was infective to sheep for a period of 44 days after subsidence of the thermal reaction. In order to test the duration of infectivity, the following experiment was carried out. Sheep 276 was infested with tick-borne fever-infected ticks, and reacted 7 days later, the reaction lasting 14 days. During the fever, blood was drawn from this animal and inoculated into a normal sheep which gave a definite reaction. Blood was drawn from No. 276 at different intervals after recovery, and tested in normal sheep, with the following results.

Fifteen days after recovery 5 c.c. of blood were inoculated into a normal sheep which developed a typical reaction. Thirty-five days after recovery, blood produced a slight temperature reaction in a normal sheep, the reaction lasting for only 4 days. Seventy days after recovery, the blood was found to be non-infective. It appears, therefore, that in this particular sheep, the blood remained infective for a period of between 35 and 70 days after recovery.

A sheep, No. 135, exposed in the field, developed a tick-borne fever reaction and recovered. Nymphs which had gorged on this sheep during the fifth week after subsidence of its reaction were bred in the laboratory, and the resulting females were allowed to infest Tup 292; this tup developed on the 8th day a temperature reaction which lasted for 8 days. The highest temperature recorded was 107·2° F. Louping-ill virus was absent from the blood when this was tested in mice on the 11th day.

It appears, therefore, that sheep which have recovered from an attack of tick-borne fever are reservoirs of infection for some considerable time, and

can infect the ticks which feed on them after their reactions are over. The alternative explanation of the reaction in Tup 292 is that the ticks acquired infection as larvae, and that the infection survived in the ticks through the nymphal stage which fed on Sheep 135 in the field. The possibility of infection lying latent through one stage of the tick has yet to be tested.

Tick-borne fever in the goat.

A goat, No. 49, was infested on the scrotum with nymphs bred from larvae which had fed on a field case of tick-borne fever. The normal temperature of the goat varies between 102 and 103° F. On the 10th day after infestation, the temperature of this goat rose to 106·6° F. The reaction lasted for 7 days, the temperature steadily subsiding during this time. By the 8th day, it was again normal (Chart IV).

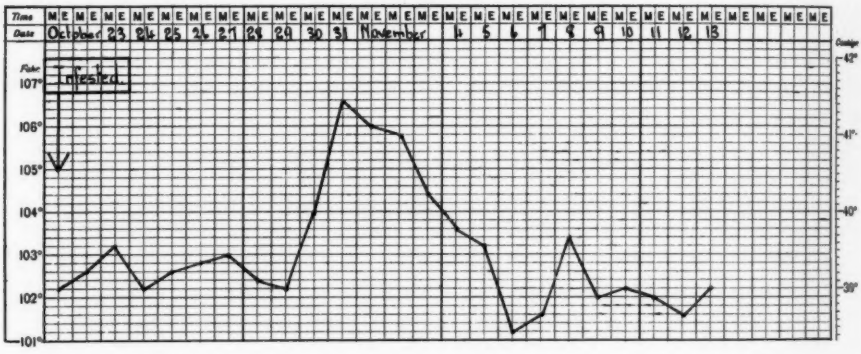

Chart IV. Tick-borne fever reaction in Goat 49 following infestation with infected nymphs.

Another goat, No. 206, was inoculated subcutaneously with 5 c.c. infective blood. On the 6th day, the temperature rose to 106° F. The reaction lasted for 6 days.

Goat No. 210, inoculated subcutaneously with 5 c.c. of infective blood, showed on the 5th day an indefinite reaction which lasted for 4 days. The temperature in this case did not exceed 103·6° F.

The goat, therefore, appears to be susceptible to tick-borne fever.

Description of the disease.

From the data given in the preceding text we may outline the chief characteristics of the disease, as it occurs after tick infestation, as follows:

Incubation period. The incubation period after infestation by infected nymphs appears to be shorter than that after infestation by females. In the former case it is usually 4–6 days but varies from 3 to 7 days. (In three cases produced by nymphal infestation, described in a previous paper (MacLeod, 1932), the periods were 3, 4 and 4 days respectively.) After infestation by

females, on the other hand, the incubation period is usually about 7–8 days, although it may vary from 5 to 13 days.

Febrile phase. After the incubation period there is an abrupt rise in temperature to between 106 and 108° F., the highest point of the thermal reaction being usually reached on the second day. The reaction is maintained for a period of from 6 to 22 days. The usual period of reaction, in the case of infection by infestation, is about 12 days. During the later stage of the reaction, the temperature gradually subsides to normal. A slight but appreciable fall preceding the initial rise in temperature is a frequent but inconstant feature of the reaction (see Charts I and II). In the case of infection by inoculation of infective blood the incubation period is shorter—usually 3–4 days—and the reaction is usually of shorter duration, the temperature falling more abruptly to normal.

Mortality. If uncomplicated by other infections, the disease seldom terminates fatally. Out of seventy-five cases produced by inoculation of infective blood, there occurred only two deaths directly attributable to tick-borne fever infection. Of the twenty experimentally produced cases of tick-borne fever in sheep recorded in this paper, two died (Tups 456 and 275). The autopsy of Tup 456 revealed no gross lesions which could be regarded as responsible for death. This sheep died apparently as a direct result of tick-borne fever infection. In the other case (Sheep No. 275), the immediate cause of death was bacillary necrosis of the liver.

Duration of infectivity of blood. The blood of a recovered case of tick-borne fever remains infective for sheep for some time after the reaction has subsided. In the case of one sheep, the blood was infective 35 days after recovery, but non-infective after 70 days. As recorded in a previous paper, blood was found to be infective when drawn 44 days after subsidence of the reaction. There is also evidence to suggest that ticks which feed on a recovered case acquire infection, and are infective in their next stage. Apparently, then, sheep which have reacted act as reservoirs of infection for some time after recovery.

Discussion.

In the course of an investigation into the transmission of louping-ill, it became apparent that the tick, *Ixodes ricinus*, was capable of causing a febrile condition in sheep. This condition was found to be due to an infective agent which could be passed from sheep to sheep in series. The disease so produced was shown to be clinically and immunologically distinct from louping-ill, and was named tick-borne fever. This paper describes the regular transmission of the disease by *Ixodes ricinus*. We have already shown that the same tick is the vector of louping-ill (MacLeod and Gordon, 1932); there is thus evidence that this tick may transmit the infective agents of two distinct diseases of sheep.

Although the mortality directly resulting from tick-borne fever is relatively low, the disease is nevertheless of considerable economic importance.

It is well known that in certain districts many sheep fail to thrive in the spring, even after there is a good growth of grass. The only clinical symptoms of illness in such sheep are dullness and loss of bodily weight. Lambs especially are said to pass through a period of unthriftiness after they are about 3 weeks old. Since this type of case occurs during the louping-ill season and is associated with the presence of ticks on the sheep, the farmer naturally attributes it to a mild infection with louping-ill. It is probable that many of these cases of so-called mild louping-ill are actually cases of tick-borne fever.

During this period on tick-infested farms it is common for a number of the sheep to die from undetermined causes, and it is possible that such deaths result from a primary infection with tick-borne fever followed by some secondary infection. Our experimental evidence (MacLeod and Gordon, 1932) shows that tick-borne fever aggravates the harmful effects of louping-ill infection, and it is probable that it may predispose sheep to other secondary infections, especially since it has a debilitating effect on the affected animal. It is therefore possible that the spring mortality which occurs among the flocks on at least some tick-infested farms may be due in many instances to secondary infections, the common predisposing cause being a primary tick-borne fever infection.

Summary.

1. The causal agent of tick-borne fever is transmitted by the adult female and the nymphal stage of the tick *Ixodes ricinus*. Two female ticks are sufficient to produce infection in a sheep. The larval stage does not appear to be infective. In a limited number of experiments the disease has not been produced in sheep by the inoculation of emulsions of presumably infective ticks.

2. The disease as produced by tick infestation is characterised by a period of incubation which varies from 3 to 13 days, after which there is a sharp rise in temperature; a "plateau" type of febrile reaction follows which lasts from 6 to 22 days, the temperature gradually subsiding to normal. Affected animals usually recover. Mortality as a direct result of infection is rare, but there is evidence to suggest that tick-borne fever may predispose affected animals to death from secondary causes.

3. The blood of a sheep which has reacted to the disease remains infective for some time after the subsidence of the thermal reaction.

4. The goat is susceptible to tick-borne fever.

5. The economic importance of tick-borne fever is discussed.

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CORRIGENDA.

Page 124, third line from below (Table I) for "Length of first 4 tarsal segments" read "Length of first tarsal segment."